EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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EMA/CHMP/476618/2011
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Kalbitor

ecallantide

Procedure No.: EMEA/H/C/002200/

Note

Day 180 Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

This should be read in conjunction with the "Question and Answer" document on the withdrawal of the application: the Assessment Report may not include all available information on the product if the CHMP assessment of the latest submitted information was still ongoing at the time of the withdrawal of the application.

7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
T□lephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7523 7455
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

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LIST OF ABBREVIATIONS

5-HT3	5-hydroxytryptamine
AAE	acquired angioedema
ACE	angiotensin-converting enzyme
AE	adverse event
AHRQ	Agency for Healthcare Research and Quality
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase (formerly serum glutamic-oxaloacetic transaminase SGOT])
AUC	area under the plasma drug concentration-time curve
$AUC_{0\text{-inf}}$	area under the plasma drug concentration-time curve extrapolated to infinity
B2	bradykinin type 2
BCIP	5-bromo-4-chloro-3-indolyl phosphate
Bid	twice daily
BLA	biologics license application
BP	blood Pressure
BUN	blood urea nitrogen
C1	complement component 1
C1q	complement component 1q
C4	complement component 4
C1-INH	C1 esterase inhibitor (also, C1 inhibitor concentrate)
%CV	Coefficient of Variation (Standard Deviation/Mean) X100
CABG	Coronary Artery Bypass Graft surgery
CBC	complete blood count
CJD	Creutzfeldt-Jakob disease
Cl	clearance
C_{max}	maximum concentration of drug in plasma
CPB	Cardiopulmonary Bypass
CRF	case report form
CTC	Common Toxicity Criteria
CTS	cardiothoracic surgery
CV	coefficient of variation
Da	Daltons
DB	Double Blind
DSMB	Data Safety Monitoring Board
DX-88	ecallantide
Dyax	Dyax Corp.
eDiary	electronic diary
EC	Ethics Committee
ECG	electrocardiogram
ECL	electrochemiluminescence
EDEMA	Evaluation of DX-88's Effects in Mitigating Angioedema
EDEMA0 (DX-88/2)	Open Label Single Ascending Intravenous Dose Study to Assess the Tolerability and Efficacy of DX-88 (Plasma Kallikrein Inhibitor) Administered Following Onset of Peripheral and/or Facial Edema or Abdominal Symptoms in Patients with Angioedema
EDEMA1 (DX-88/4)	An Ascending Four Dose Placebo Controlled Study to Assess the Efficacy and Tolerability of DX-88 (recombinant plasma kallikrein inhibitor) Administered Following Onset of Acute Attacks of Hereditary Angioedema
EDEMA2 (DX-88/5)	An Open Label Study to Assess the Efficacy and Tolerability of Repeated Doses of DX-88 (Recombinant Plasma Kallikrein Inhibitor) in Patients with Hereditary Angioedema
EDEMA3 (DX-88/14)	A Double-Blind, Placebo-Controlled Study Followed By a Repeat Dosing Phase to Assess the Efficacy and Safety of DX-88 (Recombinant Plasma Kallikrein Inhibitor) for the Treatment of Acute Attacks of Hereditary Angioedema
EDEMA3-DB (DX-88/14)	Refers specifically to the double-blind part of the EDEMA3 study EDEMA3-RD

EDEMA4 (DX-88/20)	A Randomized, Double-Blind, Placebo-Controlled, Multi-Center Study to Assess the Efficacy and Safety of DX-88 (Ecallantide) for the Treatment of Acute Attacks of Hereditary Angioedema
ELISA	enzyme-linked immunosorbent assay
Eurodis	European Organisation for Rare Diseases
FAST	For Angioedema Subcutaneous Treatment
G	gram
GCP	good clinical practice
GI	gastrointestinal
GGT	gamma-glutamyltransferase
HAE	hereditary angioedema
HCP	host cell protein
hERG	human ether-a go-go related gene
HMW	high molecular weight
HMWK	high molecular weight kininogen
HPC	High Positive control
HPLC	high-performance liquid chromatography
HPLC/MS	high-performance liquid chromatography with mass spectral detection
HR	Heart Rate
HRP	horseradish peroxidase
HSNC	human serum negative controls
IB	Investigator Brochure
ICH	International Conference on Harmonisation (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use)
ICC	intraclass correlation coefficient
ICF	informed consent form
Ig	immunoglobulin
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IMPACT	International Multi-center Prospective Angioedema C1-Inhibitor Trial
INa	inward sodium current
IQR	interquartile range
IRB	Institutional Review Board
ISE	Integrated Summary of Efficacy
ISS	Integrated Summary of Safety
Ito	transient outward potassium current
ITT	intent-to-treat
IUD	intrauterine device
IV	intravenous
IVRS	Interactive Voice Response Systems
IWRS	Interactive Web Response System
Kg	kilogram
L	litre
LACI	lipoprotein-associated coagulation inhibitor
LLOQ	lower limit of quantitation
LoOI	List of Outstanding Issues
LoQ	List of Questions
LPC	Low Positive control
LPP	longitudinal patient profiles
LTOSS	Long Term Observational Safety Study
LTS	Long Term Stability
MAA	Marketing Authorisation Application
MAb	Monoclonal Antibody
Mg	Milligram
MID	minimally important difference
Min	minute
mL	milliliter
MPA	Medical Products Agency
MRHD	Maximum Recommended Human Dose
MS	mass spectrometry

MSCS	Mean Symptom Complex Severity
MSD	meso scale discovery
NA	not available
NC	negative control
ND	not determined
ng	Nanogram (10-9 grams)
NHS	Normal Human Serum
NIAID	National Institute of Allergy and Infectious Diseases
NICE	National Institute for Health and Clinical Excellence
NOEL	no observable effect level
NS	not significant
OD	optical density
OR	odds ratio
PaCO2	partial arterial carbon dioxide pressure
PaO2	partial arterial oxygen pressure
PBS	phosphate buffered saline
PC	positive control
PDCO	Paediatric Committee
PIA	Primary Immunodeficiency Association
P pastoris	Pichia pastoris
PIP	Paediatric Investigation Plan
PK	pharmacokinetics
PO	by mouth
PP	per-protocol
PRO	patient-reported outcome
PT	prothrombin time
QA	quality assurance
QC	quality control
QRS	part of the electrocardiographic wave presenting depolarization
QT	time between of QRS complex and end of T-wave
QTc	length of time for the heart electrical system to repolarize, adjusted r
RCT	randomized controlled trial
RSD	relative standard deviation
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous
SCE	Summary of Clinical Efficacy
SCS	Summary of Clinical Safety
SD	standard deviation
serpins	serine protease inhibitors
SGOT	serum glutamic-oxaloacetic transaminase (aspartate aminotransferase[AST])
SGPT	serum glutamic-pyruvic transaminase (alanine aminotransferase [ALT])
SIR	select ion recoding
S/N	Signal-to-noise ratio
SOC	system organ class
SOP	standard operating procedure
SPA	Special Protocol Assessment
SPC	Summary of Product Characteristics
SRM	selected reaction monitoring
SSR	sum of squared residuals
SUAC	severe upper airway compromise
SUSAR	suspected unexpected serious adverse reaction
t1/2	half-life
tcalc	t-test value result
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TFPI	tissue factor pathway inhibitor
TFPI	tissue factor pathway inhibitor
tid	three times daily
tmax	time to reach maximum concentration of drug in plasma
TMB	tetramethylbenzidine
TOS	Treatment Outcome Score
TPA	tripropylamine
TT	thrombin time TXA tranexamic acid

U	unit
□g	Microgram (10-6 grams)
µg/mL	microgram/mililiter
UF/DF	ultrafiltration/diafiltration
ULOQ	upper limit of quantitation
VAS	Visual Analogue Scale
Vd	volume of distribution
WFI	water for injection

EXECUTIVE SUMMARY

Problem statement

Hereditary angioedema (HAE) is a rare disease characterized by either C1-INH deficiency (Type I HAE) or dysfunctional C1-INH (Type II HAE). The exact prevalence of HAE is unknown but worldwide it has historically been reported to be between 1 in 10,000 and 1 in 50,000. Caused by genetic mutations affecting the C1-INH gene located on chromosome 11q, HAE is inherited as an autosomal dominant trait.

The central role of plasma kallikrein in the pathogenesis of the signs and symptoms of acute attacks of hereditary angioedema (HAE) has been well established in the scientific literature.

The activity of human plasma kallikrein is normally regulated by complement component-1 esterase inhibitor (C1-INH); however, in the absence of adequate C1-INH activity, the activation of plasma kallikrein is largely unopposed. This leads to the characteristic acute attacks of HAE: episodes of swelling affecting any part of the body, including the abdominal viscera (which can result in episodes of pain, nausea, and vomiting), and the oropharynx and larynx (which can result in airway obstruction, asphyxiation, and death). These acute attacks are episodic, unpredictable, and have highly variable symptom patterns and severity both within and between patients.

HAE frequently presents in the second decade of life (adolescence) but presentation in children less than 12 years is not uncommon. While affecting both genders, HAE attacks are more frequent in females; this gender difference in frequency, in conjunction with the increased occurrence after puberty and sometimes diminution in the elderly, suggests a hormone-influenced mechanism. Mortality (estimated at 30% in undiagnosed patients) is most commonly caused by asphyxiation due to laryngeal oedema, which occurs at least once in the lifetime of approximately half of all HAE patients.

HAE attacks can be induced by a variety of stimuli or triggers, including stress, medical procedures, dental work, and hormonal changes. Although most HAE patients can identify one or more possible triggers of some of their attacks, many attacks occur without a known precipitating factor.

The frequency of HAE attacks ranges from less than 1 per year to greater than 26 per year, with some patients reporting more than 100 HAE attacks per year; on average, untreated patients have attacks every 7 to 14 days.

Consensus-based algorithmic approaches to HAE patient management identify three treatment objectives:

1) long-term prophylaxis to prevent or reduce the frequency and severity of attacks over time
2) short-term prophylaxis in advance of elective medical or dental procedures
3) treatment for acute attacks

The currently approved therapies for the treatment for the treatment of acute attacks in EU are:

1) C1-INH replacement therapies (Berinert, Cinryze and Ruconest)
2) icatibant (Firazyr), a bradykinin type 2 (B2) receptor antagonist;
3) other traditional agents including tranexamic acid, a lysine analogue antifibrinolytic agent, androgenic steroids such as danazol, and fresh frozen plasma. These agents each address a different component of the cascading pathway underlying an acute HAE attack.

The availability of an additional SC therapy for HAE would provide an advantage in terms of the available therapy option for patients.

About the product

DX-88 is intended for subcutaneous administration for the symptomatic treatment of acute attacks of hereditary angioedema in patients 12 years of age and older. The product is an orphan drug, with designation granted in 2002 (Designation Number EU/3/02/126).

The active substance is ecallantide, a recombinant protein expressed in Pichia pastoris. Ecallantide protein is comprised of 60 amino acid residues with three intramolecular disulfide bonds. Ecallantide inhibits human plasma kallikrein, binding plasma kallikrein reversibly with a rapid on-rate and a slow off-rate that results in high affinity inhibition.

The mass of ecallantide as determined by electro-spray-mass spectrometry (ES-MS) is 7054 Daltons, which is consistent with the predicted molecular weight of 7054 Daltons. Disulfide bond mapping of ecallantide confirms the expected intramolecular disulfide bond arrangement; free sulfhydryl groups are not observed. Glycosylation with one, two or three O-linked mannose units occurs at low frequency on Ser5 and Thr58. The low value for the molar ratio of total mannose to ecallantide (0.06) is indicative of the low levels of glycosylated product-related substances (PRS) in active substance.

The sequence of ecallantide was identified through the iterative selection and screening of phage display libraries containing variants of the first Kunitz domain (K1) of the naturally occurring human protein tissue-factor pathway inhibitor (TFPI), also known as lipoprotein-associated coagulation inhibitor (LACI). The amino acid sequences of ecallantide and TFPI-K1 differ by seven amino acids, five that are associated with the high affinity binding and inhibition of plasma kallikrein, and two that were intentionally added to ecallantide for efficient secretion from yeast.

The medicinal product is manufactured by sterile filtration of the active substance solution followed by aseptic filling and is a clear and colourless solution. The primary packaging comprises of Vial 2 mL filled with 1 mL of ecallantide drug product with a target overfill of 0.15 mL to allow the correct volume to be extracted.

The mechanistic trigger for the initial activation of plasma kallikrein in patients is unknown at present, but the end result is cleavage of high molecular weight kininogen by kallikrein with the release of bradykinin. Bradykinin acts on the vasculature to increase capillary and endothelial permeability, resulting in extravasation of fluids producing the pathognomonic signs and symptoms of HAE attacks.

By directly inhibiting plasma kallikrein, ecallantide reduces the conversion of HMW kininogen to bradykinin and thereby treats symptoms of the disease during acute episodic attacks of HAE.

The proposed indication for ecallantide is the symptomatic treatment of acute attacks of hereditary angioedema (HAE) in patients 12 and older.

The recommended dose of DX-88 is 30 mg (3 ml), subcutaneously administered by a healthcare professional in three 10 mg (1 ml) injections. If the attack persists, an additional dose of 30 mg may be administered within a 24 hour period. The posology is proposed to be the same in adults and children 12 years of age and older. DX-88 is not intended for self administration. In the earlier clinical studies ecallantide was administered intravenously in doses up to 80 mg and 40 mg/m2.

The proposed method of administration is with 3 SC injections each of 1 ml solution containing ecallantide 10 mg/ml.

The development programme/Compliance with CHMP Guidance/Protocol Assistance

CHMP Protocol Assistance on quality aspects was sought for the comparability program (see Annex 514 to the application form). The data presented show that CHMP advice has been followed for quality. Comparability data are provided for 2nd generation and commercial batches of drug substance and, as discussed in the Protocol Assistance. Comparability data are also provided for drug product batches manufactured at different scales and at different sites.

During development Protocol Assistance was requested by the Applicant on the non-clinical development of ecallantide. The first final advice letter was issued on 23 April 2004 (procedure no. EMEA/H/SA/468/1/2004/PA/I). Regarding non-clinical issues advice was provided on non-clinical comparability and the overall summary. In a subsequent advice issued on 17 February 2005 (procedure no. EMEA/H/SA/468/2/2004/PA/III) further advice was provided on the use of aPTT as a pharmacodynamic marker of ecallantide activity in normal humans and animals and the validation of the rat as toxicology species for ecallantide

For the clinical development CHMP protocol assistance was obtained in April 2004, CHMP Protocol Assistance in February 2005 and protocol assistance follow-up official position in September 2005.

The CHMP final advice on outcome measures for assessment of efficacy recommended that the primary endpoint be based on "time to response" analysis (time to onset of improvement, time to significant improvement and time to resolution) to assess the speed and durability of response compared with placebo, as this was considered the most clinically relevant endpoint. This was not performed as a primary endpoint by the company.

In addition SA from National agencies, FR in August 2002, SE June 2007, FR July 2007, UK July 2007, and pre-submission meeting were held with the Rapporteurs (UK December 2008 and NL February 2009).

General comments on compliance with GMP, GLP, GCP

GMP certificates have been provided for the drug substance manufacturer, the drug product manufacturer and the QP release sites. A product specific inspection is therefore not required.

A QP declaration to certify that release testing of drug substance and drug product are performed in accordance with GMP has been provided. Confirmation has been supplied that final QP release is performed to GMP.

GLP compliance was generally satisfactory. The pivotal toxicology studies were performed in GLP certified testing facilities. Some bioanalytical studies as part of toxicokinetic evaluation were not performed under GLP (e.g. ELISA of ecallantide), but good scientific standards were applied. Some test facilities where the bioanalytical studies were performed could not be traced in a listing of GLP inspected testing facilities provided by the Netherlands competent authority (VWA). However, no relevant deviations were encountered in these reports. Therefore, an audit of any study is not considered necessary.

There were minor deviations from protocols in toxicity studies which are accepted not to have had a significant impact on the ability to determine the safety profile of ecallantide. Some safety studies were not conducted in compliance with GLP, typically because they were exploratory studies where there

was an intent to have further data in compliance with GLP. The availability of data from other studies that are GLP-compliant is sufficient reason not to raise objections to these instances of lack of GLP-compliance.

The company states that all trials were conducted according to the principles of GCP as specified in the appropriate regulations (CPMP/ICH/135/95) and were reviewed and approved by institutional review boards or ethics committees, as appropriate.

Type of application and other comments on the submitted dossier

- Legal basis
- This is a Centralized Application in accordance with Article 6 of Regulation (EC) No 726/2004 and Article 8(3) of Directive 2001/83/EC, as amended, for a Community Marketing Authorization.
- Ecallantide, as "recombinant inhibitor of human plasma kallikrein," is designated as an orphan medicinal product for the treatment of angioedema, entered into the Community register under the number EU/3/02/126 (December 2002).
- Conditional approval

  N/A
- Approval under exceptional circumstances

  N/A
- Accelerated procedure

  N/A
- Biosimilarity

  N/A
- 1 year data exclusivity

  N/A
- Significance of paediatric studies

With respect to the Paediatric Regulation, the European Medicines Agency granted ecallantide solution for injection for subcutaneous use a product-specific waiver for children from birth to less than 2 years and the above mentioned condition, on the grounds that the specific medicinal product does not represent a significant therapeutic benefit, as clinical studies are not feasible. In addition, a deferral for the conduct of clinical study in prepubertal patients was granted. The study, a 3-Part Study to evaluate PK, Safety and Efficacy of SC ecallantide in Prepubertal Paediatric Patients Experiencing Acute Attacks of HAE is planned to be initiated by November 2010 and completed with the last patient, last visit by November 2014. (8 February 2010 [EMA/74199/2010, P/19/2010]).

PDCO advised that from studies EDEMA1, EDEMA2, EDEMA3, EDEMA4 (submitted with this MAA) and DX-88/19 (currently ongoing) at least 25 separate patients below the age of 18 should be included in the five studies; of these, at least 4 should be younger than 12 years. At least 16 patients below the age of 18 should be included in the double-blind, placebo-controlled studies.

Efficacy and safety results in pediatric patients must be reported separately and by age categories. Individual data must be provided, PK data analysis per age categories and in comparison to adults must be detailed.

With the compliance check of the Paediatric Investigation Plan (EMA/270322/2010, 21 may 2010) one comment was made by the PDCO: the PK data per category should be detailed (no blocking point).

SCIENTIFIC OVERVIEW AND DISCUSSION

Quality aspects

Kalbitor (DX-88) is intended for subcutaneous administration for the symptomatic treatment of acute attacks of hereditary angioedema in patients 12 years of age and older. The product is an orphan drug, with designation granted in 2002 (Designation Number EU/3/02/126).

The active substance is ecallantide, a recombinant protein expressed in Pichia pastoris. Ecallantide protein is comprised of 60 amino acid residues with three intramolecular disulfide bonds. Ecallantide inhibits human plasma kallikrein, binding plasma kallikrein reversibly with a rapid on-rate and a slow off-rate that results in high affinity inhibition.

The sequence of ecallantide was identified through the iterative selection and screening of phage display libraries containing variants of the first Kunitz domain (K1) of the naturally occurring human protein tissue-factor pathway inhibitor (TFPI), also known as lipoprotein-associated coagulation inhibitor (LACI). The amino acid sequences of ecallantide and TFPI-K1 differ by seven amino acids, five that are associated with the high affinity binding and inhibition of plasma kallikrein, and two that were intentionally added to ecallantide for efficient secretion from yeast. The difference between the natural TFPI and ecallantide is part of major objection one, because of possible relation with immune responses observed against ecallantide in the clinical studies.

The medicinal product is manufactured by sterile filtration of the active substance solution followed by aseptic filling and is a clear and colourless solution. The primary packaging comprises of Vial 2 mL filled with 1 mL of ecallantide drug product with a target overfill of 0.15 mL to allow the correct volume to be extracted.

Drug substance

The active substance is ecallantide, a recombinant protein expressed in Pichia pastoris. Ecallantide protein is comprised of 60 amino acid residues with three intramolecular disulfide bonds. The primary structure of ecallantide is adequately described. Details of the derivation of the sequence are provided. The general properties of ecallantide, including binding capability and glycosylation have been appropriately described. Some information is provided on secondary and tertiary structure and the relationship with mechanism of action, however concerns regarding amino acid changes and immunogenicity to ecallantide remain in part.

GMP certificates have been provided for drug substance and drug product manufacturers but certificates for the recently inspected drug substance storage sites and QC testing sites are requested.

The drug substance manufacturing process consists of inoculum preparation, seed fermentation, production fermentation with an induction phase, dilution, chromatography, ultrafiltration and diafiltration, filtration and storage.

Details of operating parameters, IPCs and CIPCs have been provided. The Applicant has agreed to tighten the resin/membrane loads once the re-use studies are completed. The criteria and definitions used when grading process controls have been clarified. The Company has provided a clear definition of when an IPC is considered critical and include justification for the strategy applied to grade the different control parameters. Control of excipient composition of drug substance and drug product is satisfactory.

Details of the raw materials used in fermentation are appropriate. The choice of P. pastoris as the production strain has been explained. Development of the plasmid vector and ecallantide sequence is adequately described and full sequencing of the plasmid has been conducted. The effect of the 5 amino acid changes on the secondary and tertiary structure of ecallantide is explained. This was raised as part of the major objection relating to immunogenicity to DX-88.

The preparation and testing of master seeds and working seed banks have been described. The qualification of future WCB has been detailed and is considered acceptable.

Studies have been conducted to provide information on copy number, insertion site and genetic stability of the ecallantide sequence. The additional bands seen on Southern analysis are attributed to sample degradation or incomplete cleavage.

Full data from process validation studies have been provided and include descriptions of the purpose of each manufacturing step. Despite the wide limits for IPC and process validation parameters applied to the process validation studies, the purification steps are consistent for the parameters tested. The absence of viral inactivation/removal studies of the process is considered acceptable.

Data to demonstrate consistent removal by the process of process-related impurities have been provided. However, the levels of HCP levels in the product cannot be considered acceptable based on clinical studies. This issue was raised as a part of major objection 2 and remains unresolved.

Data to demonstrate stability of process intermediate pools and filter validation data are acceptable.

The Applicant proposes to confirm the results of small scale resin reuse studies with concurrent validation at the manufacturing scale, justification for the proposed parameters and acceptance criteria are provided. Levels of leachables/extractables are considered acceptable. Shipping validation data are acceptable.

Descriptions of changes to the manufacturing process during development are satisfactory and lists are provided to indicate which batches, derived from which manufacturing process, were used in non-clinical and clinical studies. Comparability data have been provided and shows comparability of all quality attributes other than HCP and potency. HCP levels have been reduced during process development, however, batches used in the pivotal clinical study had lower HCP levels than commercial scale batches.

A thorough characterization of ecallantide, ecallantide-related substances and host-related impurities has been conducted. Ecallantide drug substance consists of ecallantide and product-related substances (PRS) and is highly pure. O-linked glycosylation can occur with low frequency on Ser5 and Thr58 with the attachment of one, two, or three mannose units. Different PRS forms have been identified and purified, and their structures and activity have been elucidated. Of particular interest are the glycosylated forms of the product which may have implications for the safety of the product. This issue was raised as a part of a major objection relating to immunogenicity of DX-88 in clinical trial patients and is now considered resolved. In addition, the extinction coefficient used to determine protein concentration was changed during development and this change may have impacted the protein content of final product. The Applicant has provided a satisfactory explanation of the change of extinction coefficient.

Specifications are adequately listed. Drug substance release and shelf-life specifications are the same except that HCP and bioburden testing are not performed as part of drug substance stability studies.

Questions have been raised in many sections of the dossier (characterization, process validation, specifications and analytical methods) resulting from the change of potency assay at a relatively late stage of development.

The Applicant now intends to use both the the 2nd-Generation Activity Assay, in which ecallantide activity is expressed as Drug Inhibitory Units (DIU) related to Reference Standard (%RSA), and the Inhibition Constant Ki Assay in the specification for drug substance and drug product. This is acceptable however, further clarification and validation data in line the pharmacopoeial guidance is required. Furthermore drug substance and drug product specifications for potency remain wide and further justification has been requested. The acceptance criterion for HCP cannot be considered acceptable when clinical data are considered.

Details of analytical test methods and SOPs for the potency assay, monosaccharide assay and HCP assay have been provided. Validation reports for the potency assay (including any data available for potency assays used during development), monosaccharide assay and HCP assay have been provided. Some points are raised regarding the validation of the potency assay and the reintroduced quality assay (2^{nd} generation Activity Assay).

The batch data provided indicate that all batches met the specifications in use at the time and demonstrate consistency of the commercial manufacturing process. Batch failures and corrective measures have been described.

Specifications other than potency, have been set appropriately although questions remain relating to potency. The Applicant has justified the removal of certain tests used during development.

Batch data have been provided for the commercial reference standard and for the clinical reference batch.

The proposed container closure system for drug substance is acceptable. Stability data support the use of the container closure system.

The proposed commercial shelf life for ecallantide drug substance is 36 months at -20 °C± 5 °C. The proposed shelf life is supported by stability data from 6 primary stability batches (manufactured at the commercial scale) stored at -20 oC or 5 oC for 24 or 36 months. The primary stability indicating quality parameters are identified. The post-approval stability protocol is acceptable.

Drug Product

The drug product is a sterile, preservative-free isotonic solution with an ecallantide concentration of 10 mg/mL provided in a 2 mL glass vial. Each vial is filled with 1 mL. The manufacture of drug product involves sterile-filtration of the drug substance and filling into vials. There is no dilution or excipient addition in drug product manufacture and no dilution prior to administration.

The formulation has been maintained throughout development. Vials are overfilled by 0.15 ml to ensure that a full dose of product can be administered.

The drug product manufacturing process consists of sterile filtration and aseptic filling of ecallantide drug substance into vials; no dilution or addition of excipients occurs during drug product manufacture. Full descriptions of each stage of the manufacturing process have been provided.

The main changes during development of drug product manufacturing process were the filling scale and manufacturing site. Comparability data for the different manufacturing sites and scales have been provided. Appropriate GMP certificates have been provided for all drug product manufacturing sites.

Process controls and in-process tests are provided. Operating parameters, IPCs and CIPCs are acceptable.

Process validation studies have shown that the process is consistent across the range of batch sizes and that drug product is homogenous. Further potency data are however requested. Pooling of batches of drug substance for the manufacture of a drug product batch has been shown to be acceptable. Shipping validation data are acceptable.

The drug product specifications are in accordance with Ph Eur 2008:0520. Further justification for the specification for potency, ideally based on batches used in phase 3 clinical trials and dose finding studies has been requested. The acceptance criterion for the product related substance should be justified at release and at drug product expiry.

Analytical methods are those used for drug substance or are Ph Eur methods.

Batch data are provided however, these batches have been analysed using an old version of the potency assay, rather than using the assay for which assay validation data are available and which will be used for commercial product.

Specifications are based on statistical analysis of both drug substance (both scales) and drug product batches. Drug product specifications for HCP can not be considered acceptable given the immunogenicity seen in clinical trials.

The container closure system is adequately described. Specifications and the testing regimen are appropriate.

The proposed commercial shelf life for ecallantide drug product is 36 months at 2°C to 8°C. Further data on potency are requested. At present a shelf-life for drug product of only 24 months at 2-8 oC is acceptable because specifications have yet to be justified and data from the commercial process is not available for the 36 month time-point. The SPC should be revised accordingly. Data from photostability studies have not been provided and are requested. Data from thermal cycling studies have been provided, and the SPC has been revised to remove reference to storage at room temperature.

The Applicant claims that any animal viruses introduced during the manufacture of the master seed by the use of porcine digest of bovine trypsin would be inactivated by the low pH at the fermentation induction step and has provided a relevant risk assessment based on dilution factor.

Discussion on chemical, pharmaceutical and biological aspects

The safety profile of ecallantide is dominated by its high immunogenicity, both for IgE and IgG antibodies. Two quality parameters are identified in relation the immunogenicity of ecallantide.

The Applicant's response indicates that immunogenicity is an intrinsic property of ecallantide being non-self to the human immune system this is attributed to the amino acid changes introduced to the TFPI Kunitz domain sequence and is an intended feature of the drug substance which cannot be resolved. The question remains as to whether the drug substance is therefore inherently immunogenic resulting in, or contributing to, the anaphylactic/anaphylactoid reactions seen in clinical trials An acceptable level of immunogenicity and hypersensitivity reactions is part of the overall benefit-risk assessment.

The Applicants suggests that there is no correlation between hypersensitivity and IgE antibodies. That notion is not shared. The group of patients with hypersensitivity reactions has a significantly higher percentage with IgE antibodies against HCP or ecallantide. The fact that higher HCP batches are associated with higher immunogenicity as stated by the Applicant indicates that the observed (IgE) immunogenicity is not only an intrinsic property of ecallantide. If the current reduction in HCP has

reduced the seroconversion rate there is no theoretical argument that this could not be further reduced by reducing the HCP. A further reduction of the HCP is likely to improve the safety profile and this should be pursued by the Applicant. A contribution of ß-glucans to the immunogenicity and hypersensitivity reaction (HSR) cannot be ruled out and should be further investigated. It is unlikely that antibodies against ecallantide cross-react with TFPI.

Though the Applicant has provided an explanation of the development and function of the potency assays used. The potency has been executed in two different ways during development. Issues on validation of the assay and bridging between the assays have not sufficiently been addressed. Further clarification is required before these assays can be accepted.

Most issues concerning process control and quality of the drug substance and drug product have been satisfactorily addressed.

Conclusions on the chemical, pharmaceutical and biological aspects

Based on the review of the data on quality the CHMP considers that the application for DX-88 **is not approvable**, as major deficiencies remain, which preclude a recommendation for marketing authorisation.

The Applicant should note that the Quality Major Objections can only be considered to have been satisfactorily resolved when the related clinical major objections have been resolved.

The most important quality deficiencies relate to the development of antibodies to ecallantide and to host cell protein in clinical trial patients treated with this product. The applicant has indicated that immunogenicity is an intrinsic property of ecallantide being non-self to the human immune system due to the amino acid changes introduced to the TFPI Kunitz domain sequence and cannot be resolved as a quality issue. The question remains as to whether the drug substance therefore inherently results in, or contributes to, the anaphylactic/anaphylactoid reactions seen in clinical trials.

The question on the acceptability of the level of these adverse events is referred to the clinical assessment. Thus, the quality of the drug product can not be considered acceptable.

Non clinical aspects

Pharmacology

Ecallantide was shown to inhibit human plasma kallikrein with potency in the picomolar range and to be similarly active against this target in cynomolgus monkeys, but to be much less potent against rat and mouse plasma kallikrein. Despite being less potent in rodents, ecallantide showed relevant pharmacological activity to inhibit bradykinin-mediated effects in rats and in mice; adequate evidence of the primary mechanism of action has been presented. In humans, off-target effects could arise from inhibition of plasmin and from Factor XIa when comparing Ki values and the concentrations measured when 30 mg ecallanditde is given subcutaneously to humans. No particular risks of thrombosis or excessive bleeding was identified in animal tests, but this should be viewed cautiously as animals may not be similarly sensitive to these off-target effects. The applicant did not initially discuss limitations on assessing the effects in animals of ecallantide at these two targets and was asked to do so. The applicant responded and it is concluded that this aspect of the preclinical dataset has been adequately considered.

Safety pharmacology studies identified possible effects on the cardiovascular system, specifically the possibility that intravenous doses higher than those that can be tolerated when given subcutaneously might be associated with ventricular arrhythmias and death. Apart from this finding, the safety pharmacology studies did not suggest any other particular reason for concern. The effect was occasionally present in rats dosed at 25 mg/kg but it was not seen in cynomolgus monkeys given ecallantide intravenously. As the human dose is 30 mg (~0.5 mg/kg) and this is given subcutaneously, it is judged that there is a reasonable safety margin and that this finding is not a relevant risk for the proposed human dosing. The applicant noted that bradycardia may predispose to delayed ventricular repolarisation and also noted that ecallantide could inhibit Factors VIII, IX, XI and XII coagulation in haemophiliacs. As these considerations are based on extrapolations from *in vitro* testing, there is insufficient evidence to include warnings of these effects in the SPC, based on the preclinical data.

Pharmacokinetics

Several methods were developed and applied to the quantification of ecallantide in biological samples from animals. Mostly, ecallantide was quantified by ELISA methods. The methods used to quantify ecallantide and to detect anti-ecallantide antibodies are accepted as suitable. Pharmacokinetic data from animals suggested that the drug is rapidly eliminated with none detected in blood from ~6 hours after a single dose. T max when given subcutaneously was variable but data showing Tmax of <1 hour were consistently generated. Once a day dosing in animals would not ensure continuous exposure over a 24 hour period.

Toxicology

The studies in rats and monkeys are of adequate design to conclude that general toxicity of ecallantide has been adequately studied. Additional studies were also done in minipigs. In the general toxicity programme, the major findings were as follows:

- prolongation of aPTT, but no indication of bleeding
- instances of arrhythmia, with some unexplained deaths
- local intolerance of subcutaneous injections of concentrations higher than that intended to be used clinically

Prolongation of aPTT is expected based on inhibition of plasma kallikrein and this showed reversibility on elimination of ecallantide from the blood. An approximate lethal dose was identified in rats of 25 mg/kg intravenously; no deaths occurred following subcutaneous dosing. Rabbits may be more sensitive as death was seen at 5 mg/kg intravenously. The possibility that the sudden deaths in rats was due to allergic reactions was considered but the clinical picture did not reflect this, and in instrumented animals given the same doses but who survived, ventricular arrhythmias were detected, suggesting that these events probably are of cardiac origin. Local intolerability was the dose-limiting toxicity with subcutaneous dosing. The dose and concentration needed for this effect was such as to suggest that this is not a relevant concern at the human dose.

Longer term toxicity studies used dosing every 2 or 3 days. In the species that responded pharmacodynamically to ecallantide in a manner quantitatively similar to humans, the cynomolgus monkey, there is clear evidence of a suitable safety margin. Prolonged bleeding or thrombosis did not occur in animals given high doses of ecallantide (23-25 mg/kg, compared to a dose of ~0.5 mg/kg in humans).

In the toxicity studies, there was an unusual response on repeated dosing in that antibodies to ecallantide were formed which were neither inhibitory (aPTT remained prolonged in their presence indicating lack of blockade of pharmacodynamic activity) nor which accelerated clearance. Elimination was prolonged in the presence of anti-ecallantide antibodies. The applicant attributes this possibly to the size of molecule formed by ecallantide and anti-ecallantide antibodies being too great to undergo renal filtration and elimination.

Decreases in sperm count were detected but this did not affect male reproductive capacity. The reproductive toxicity testing in pregnant animals suggested quite a different profile depending on the route of administration, with local tolerability concerns limiting the dose that could be given subcutaneously such that toxicity in pregnant animals seen when ecallantide was given intravenously was not seen when it was given subcutaneously. When given subcutaneously, the intended clinical route, near maximally tolerable doses caused no reproductive toxicity. Comparisons of exposure at the NOEL dose of 20 mg/kg subcutaneously with that of human indicates a clear margin, but this is compromised by the different sensitivity of rats to ecallantide. When given intravenously, rats, but not rabbits, showed fetal toxicity at doses which also caused toxicity in maternal animals. The applicant proposes that these data be reflected in the SPC.

The absence of genotoxicity testing is acceptable and in accordance with international guidelines: ecallantide is a peptide and guidance reflects that there is no expectation of any direct interaction with DNA or other chromosomal material. For the assessment of carcinogenic potential, a rodent study could be viable. Guidance suggests that the duration of clinical dosing should influence the need for assessment of carcinogenic potential: it is likely that each use of this product will be short, but that there will be multiple treatments over a long period of time, as treatment is not curative. No potential for proliferation of transformed cells or for clonal expansion has been identified in preclinical testing with ecallantide. In 2004, the applicant's preclinical development plan was the subject of EMA Protocol Assistance and areas of weakness were identified at that time, but this did not include the absence of a carcinogenicity study; this position, (ie of not identifying a need for carcinogenicity studies when asked if the preclinical data are adequate) was maintained in national agency meetings too. Finally, ICH guidance on the need for carcinogenicity studies indicates that continuous use for at least 6 months merits consideration of a carcinogenicity study. This is not met by the intended use of ecallantide and the absence of a rodent lifetime carcinogenicity study can be agreed.

Ecotoxicity/environmental risk assessment

CHMP guidance (Guideline on the environmental risk assessment of medicinal products for human use EMEA/CHMP/SWP/4447/00) exempts peptides from environmental risk assessment as they considered unlikely to result in significant risk to the environment.

Discussion on non-clinical aspects

The data presented are generally adequate to establish the mechanism of action of ecallantide in this indication. Although rodents were pharmacodynamically less sensitive to the drug, the cynomolgus monkey was approximately as sensitive to the drug as humans, based on *in vitro* testing. Toxicity identified was of a prolongation of aPTT but no evidence of excess bleeding, either in rats or monkeys, effects on the electrical activity of the heart in rats which was the probably cause of death at the minimally lethal dose in rats (25 mg/kg, intravenously) and of local intolerance of large amounts injected subcutaneously. Reproductive toxicity was seen when given intravenously but when given subcutaneously, maximally tolerable doses did not affect reproductive function.

Conclusion on non-clinical aspects

In conclusion, there are no major objections to approval based on the non-clinical review.

Clinical aspects

Tabular overview of clinical studies

Study ID Phase	Design and Control Type	Study Objective	Study Drug and Dose Control Drug Route and Regimen	Subjects by Arm Completed/ Entered	Duration of Treatment	Study Population Inclusion Criteria
Completed HAE Studies						
DX-88/15 Phase 1	Double-blind, randomized, crossover Placebo	Safety and Bioequivalence	Ecallantide (liquid) 30 mg as 3 1-mL SC injections and placebo (lyophilized) as 1 1-mL SC injection OR Ecallantide (lyophilized) 30 mg as one 1 mL SC injection and placebo (liquid) as 3 1-mL SC injections	12 subjects each 23/24	2 doses (7 days apart)	Healthy subjects
DX-88/1 Phase 1	Double-blind, escalating single-dose Placebo	Safety and Pharmacokinetics (PK)	Ecallantide 10, 20, 40, or 80 mg IV	10 mg: 2 20 mg: 2 40 mg: 4 80 mg: 4 Placebo: 4 16/16	Single dose	Healthy subjects
DX-88/6 Phase 1	Open-label, repeat-dose N/A	Safety and PK	Ecallantide 20 mg/m2, 10 min IV, 3 weekly doses 20 mg/m2, 4 hr IV, 1 dose IV	Dose 1: 8 Dose 2: 8 Dose 3: 7 Dose 4: 6 6/8	4 doses (7 days apart)	Healthy subjects

Study ID Phase	Design and Control Type	Study Objective	Study Drug and Dose Control Drug Route and Regimen	Subjects by Arm Completed/ Entered	Duration of Treatment	Study Population Inclusion Criteria
DX-88/13 Phase 1	Open-label, repeat-dose, crossover Active	Safety and PK	Ecallantide 30 mg 10-minute IV 10 mg SC injection and two 1 mL placebo SC injections 30 mg SC Once-weekly, cross-over	6 subjects each 16/18	3 doses (7 days apart per dose)	Healthy subjects
DX-88/2 EDEMA0 Phase 2	Open-label, escalating, single-dose	Safety, efficacy and PK	Ecallantide 10, 40, or 80 mg IV Single-dose IV	10 mg: 3 40 mg: 3 80 mg: 3 9/9	Single dose	HAE or AAE Age 18 or older presenting within 10 hours of onset of attack
DX-88/4 EDEMA1 Phase 2	Double-blind, escalating, single-dose Placebo	Safety, efficacy and PK	Ecallantide 5, 10, 20 or 40 mg/m^2 Placebo Single-dose IV	5 mg/m^2 – 10 10 mg/m^2 – 10 20 mg/m^2 – 10 40 mg/m^2 – 11 placebo – 8 49/49	Single dose	HAE Age 10 or older onset of an acute attack of HAE considered at least moderately severe, present within 4 hours of onset
DX-88/5 EDEMA2 Phase 2	Open-label, ascending, repeat-dose	Safety, efficacy and PK	Ecallantide 5, 10, 20 mg/m^2 IV; 30 mg SC IV and SC	5 mg/m^2 IV – 18 10 mg/m^2 IV – 55 20 mg/m^2 IV – 9 30 mg SC – 31 77/77	Multiple doses (≥7 days apart per dose)	HAE Age 10 and older confirmed diagnosis of HAE, acute, at least moderately severe attack, present within 4 hours of onset

Study ID Phase	Design and Control Type	Study Objective	Study Drug and Dose Control Drug Route and Regimen	Subjects by Arm Completed/ Entered	Duration of Treatment	Study Population Inclusion Criteria
DX-88/14 EDEMA3-DB Phase 3	Double-blind, single-dose	Efficacy and safety	Ecallantide 30 mg and placebo SC Single-dose SC	36 patients each arm 71/72	Single dose	HAE, Type I or II Age 10 and older, documented diagnosis, moderate or severe HAE attack , present within 8 hours of onset
DX-88/14 EDEMA3-RD Phase 3	Open label, repeat-dose	Efficacy and safety	Ecallantide 30 mg SC	67 patients 66/67	Multiple doses (≥72 hours apart per dose)	HAE, Type I or II Age 10 and older, documented diagnosis, moderate or severe HAE attack , present within 8 hours of onset
DX-88/20 EDEMA4 Phase 3	Double-blind, single dose followed by possible open-label dose for severe upper airway compromise, incomplete response, or relapse	Efficacy and safety	Ecallantide 30 mg SC Placebo 30 mg SC	48 patients each (double-blind) 95/96 37 patients (open-label)	Single double-blind dose: Single open-label dose for severe upper airway compromise, incomplete response, or relapse	HAE, Type I or II Age 10 and older, documented diagnosis, moderate or severe HAE attack, present within 8 hours of onset

Study ID Phase	Design and Control Type	Study Objective	Study Drug and Dose Control Drug Route and Regimen	Subjects by Arm Completed/ Entered	Duration of Treatment	Study Population Inclusion Criteria
Ongoing HAE Studies						
DX-88/19 Phase 3	Open-label, repeat dose	Efficacy and safety	Ecallantide 30 mg SC, 2 or 3 injections, not to exceed 1.7 mL per injection	Up to 150 patients eligible for enrollment 0 completed/148 treated	Multiple doses (≥72 hours apart per dose)	HAE, Type I or II Age 10 and older, documented diagnosis, present within 8 hours of onset
DX-88/24 Phase 4	Open-label, repeat dose	Long-term safety	Ecallantide 30 mg in accordance with commercial use Skin test, percutaneous and inradermal. Various dilutions; test dose, SC 10mg	200 patients : 150 ecallantide naïve and 50 non-naïve 0/0	Multiple doses	HAE patients
Completed Cardiothoracic Surgery Indication						
DX-88/3 Phase 2	Double-blind, ascending dose Placebo	Safety and PK	Ecallantide 30, 60, or 120 mg Placebo 3 perioperative stages (IV, bolus, IV)	30 mg: 11 60 mg: 11 120 mg: 9 Placebo: 11 37/42	Single dose	CTS patients

Additional studies for safety and efficacy have also been done for the cardiothoracic indication.

Type of Study	Study Identifier	Location of Study Report	Objective(s) of the Study	Study Design and Type of Control	Test Product(s), Dosage Regimen, Route of Administration	Number of Subjects by Arm Completed / Treated	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status, Type of Report
CARDIOTHORACIC SURGERY (CTS) INDICATION									
Safety	DX-88/3	5.3.5.4	Safety and PK	Double-blind, ascending dose Placebo	Ecallantide 30, 60, or 120 mg Placebo 3 perioperative stages (IV, bolus, IV)	30 mg: 11 60 mg: 11 120 mg: 9 Placebo: 11 37/42	CTS patients	Single dose	Complete, full
Efficacy	DX-88/16 (KALAHARI 1)	5.3.5.4	Safety, efficacy, and PK	Double-blind Placebo	Ecallantide 15 mg or 91 mg IV	15 mg: 26 91 mg: 25 Placebo: 18 64/69	CTS patients	Single dose	Complete, CSR body
Efficacy	ECAL-PCPB-08-02 (CONSERV-1)	N/A	Safety and Efficacy	Double-blind Placebo	Ecallantide IV loading and IV infusion, appx: Low: 5 mg Mid: 25 mg High: 75 mg Placebo	Low: 62 Mid: 59 High: 65 Placebo: 63 TBD/249	CTS patients	Continuous administration for no longer than 6 hours	Terminated, report in preparation
Efficacy	ECAL-CCPB-08-07 (CONSERV-2)	N/A	Safety and Efficacy	Double-blind Active control	Ecallantide IV loading and IV infusion Appx 75 mg Tranexamic acid .60 mg/kg IV	Ecallantide: 109 Tranexamic acid: 109 TBD/218	CTS patients	Continuous administration for no longer than 6 hours	Terminated, report in preparation

Pharmacokinetics

Analytical assays. Three analytical methods, 2 HPLC/MS and 1 ELISA, have been used to detect ecallantide. The HPLC/MS methods were not sensitive enough to calculate pharmacokinetic parameters adequately. Plasma concentrations could be measured only up to 2 hours after IV administration of ecallantide. The ELISA method was very sensitive with an LLOQ 0.156 ng/ml enabling to determine the elimination accurately. Therefore, the results from studies DX-88/13, DX-88/15 and DX-88/5 (HAE patients) are more reliable.

Endogenous TFPI, which has 88% homology to ecallantide, did not interfere with the assay for ecallantide. Effect of anti-ecallantide antibodies on the detection of ecallantide has not been reported.

Immunogenicity assays. A three tier strategy, conform the NfG CHMP/BMWP/14327/06 was applied: the bridging assay with ECL detection (in early studies non-IgE ELISA) was used to screen, confirm, and titer anti-ecallantide antibodies. This assay, along with the accompanying kinetic enzyme neutralizing antibody assay, was utilized in the pivotal Phase 3 trials DX-88/14 (EDEMA3) and DX-88/20 (EDEMA4). IgE antibody assays were developed to detect antibodies to ecallantide and the host cell P. pastoris. The assays seem adequate to detect antibodies against ecallantide and against host cell P. pastoris.

In vitro enzyme inhibition measurements demonstrated that ecallantide is a potent, selective, and reversible inhibitor of human plasma kallikrein with an equilibrium inhibition constant (Ki) of 25 pM. Enzyme specificity studies demonstrated that ecallantide weakly inhibited 5 additional proteases including neutrophil elastase (Ki=0.75 µM), tissue kallikrein 2 (Ki =0.29µM), pancreatic trypsin (Ki =69 nM), plasmin (Ki =29 nM), and factor XIa (Ki=1.7 nM). Ecallantide demonstrates selectivity for plasma kallikrein over these other enzymes of between 60-fold to 30,000-fold. The maximum ecallantide concentration in HAE patients receiving a 30 mg SC dose is expected to be approximately 0.6 µg/mL or 80 nM. In a series of coagulation studies ecallantide at 1.0 ug/ml did not inhibit factor XI and only

partially (approximately 20%) inhibited plasmin. It is therefore unlikely that ecallantide would display any clinically meaningful inhibition of plasmin or factor Xia at the proposed posology of 30mg SC.

As noted above, ecallantide is a highly potent, reversible and specific inhibitor of plasma kallikrein and is more potent than C1-INH (ecallantide Ki =25pM vs C1 INH apparent Ki=5nM; [Report 650-0042; Davis 2004]) under identical assay conditions. *In vivo*, the plasma concentration (mean C_{max} = 80nM) achieved after a 30mg SC dose is proposed to be sufficient to block plasma kallikrein for the expected duration of an HAE attack without risk of symptom rebound. However whether this is the case is unclear and the clinical data do not provide evidence of convincing efficacy and also demonstrate a higher rate of AEs of "HAE" in the ecallantide group compared with placebo. This means that the applicant is required to provide further justification for the proposed posology in terms of adequacy if route, dose and length of effect.

The applicant refers to a publication of Kaufman et al (1991) that it is estimated for example that between 30 and 110nM of active enzyme is generated in an acute attack of HAE and therefore a 30 mg SC dose should be sufficient. This calculation is not comprehensible, and the applicant is asked to discuss this estimation.

Table 2: Ecallantide clinical studies contributing to clinical pharmacology

study	Population, N treated/evaluated	Study design	Product-route-dose	PK data
DX-88/1	HV, N=16/12	Phase 1, single ascending dose	Liquid - i.v. 10'-10, 20, 40, 80 mg	intensive
DX-88/2 (EDEMA0)	HAE, N= 9	Phase 2, single ascending dose	Liquid – i.v. 10'-10, 40, 80 mg	sparse
DX88/4 (EDEMA1)	HAE, N= 26	Phase 2, single ascending dose	Liquid – i.v. 10'-5, 10, 20, 40 mg/m^2	sparse
DX-88/5 (EDEMA2)	HAE, N=77	Phase 2, dose finding, repeat dosing	Liquid – i.v. 10' -5, 10, 20 mg/m^2 Liquid - SC – 30 mg	sparse
DX-88/6	HV, N=8	Phase 1, repeat dose	Liquid - i.v. 3x10' 1x240'weekly interval – 20 mg/m^2	intensive
DX-88/13	HV, N=18	Phase 1, 3-period cross-over, single, absolute bioavailability	Liquid – i.v. 10" – 30 mg Liquid – s.c. –10 mg, 30 mg	intensive
DX-88/15	HV, N=24	Phase 1, 2-period cross-over, single, bioequivalence	Liquid vs. lyophilised – s.c.- 30 mg	intensive
popPK	HV, N=62 HAE, N=35	Studies DX-88/1, DX-88/6, DX-88/13, DX-88/15, DX-88/2, DX-88/4		

The pharmacokinetics of ecallantide following IV and SC administration was evaluated in 4 studies in healthy subjects and 3 studies in patients with HAE at fixed doses ranging from 10 to 80 mg, or body weight adjusted doses ranging from 5 to 40 mg/m2. Non-compartmental PK analysis was conducted in the studies with intensive PK data in healthy subjects. These studies compared the liquid product with a lyophilized product in single and multiple dose PK studies of ecallantide and examined dose-proportionality and time dependency. A population pharmacokinetic model was developed to evaluate covariate factors.

Because ecallantide is a protein and not subject to metabolism by P450 enzymes, no metabolism studies and no study in hepatic impairment have been conducted. The presence of anti-ecallantide antibodies was tested throughout the clinical development plan.

Results from the PK studies DX-88-1, DX-88/13, the repeat dose PK study DX-88-6 and data from the population PK model are detailed below.

DX-88-1.

This was a double-blind, placebo controlled, single ascending dose study to assess the tolerability (primary objective) and the pharmacokinetic profile (secondary objective) of ascending single doses of DX-88 in healthy subjects.

The small number of subjects scheduled to receive the 10 and 20 mg DX-88 doses in this study (n = 2) make interpretation of the pharmacokinetic results difficult. In addition, due to the assay Lower Limit of Quantification (LOQ), full 24 hour plasma profiles were not obtained following all of the DX-88 doses.

A summary of the mean plasma pharmacokinetic parameters by dose level are presented in Table 3.

Table 3

	Treatment					
Parameter	10 mg DX-88	20 mg DX-88	40 mg DX-88		80 mg DX-88	
Cmax (µg/mL)	2.00	4.75	7.68		14.80	
Tmax (Hrs)	0.167	0.2085	0.1670		0.1670	
AUC (0-24h) (µg/mL h)	0.9212	3.4212	7.5322		16.3031	
AUC (0-Infinity) (µg /mL h)	1.4008	4.7085	8.8234		17.6560	
Half-life (h)	0.5545	1.1940	α phase	β phase	α phase	β phase
			0.7570	1.5547	1.2800	1.7115
Lambda Z	1.2521	0.5822	0.4461		0.4109	
Clearance (mL/h)	7309.8	4257.2	4559.5		4574.6	
Apparent Volume of Distribution (mL/h)	5871.8	7350.7	10209.5		11177.9	

DX-88, when administered as IV infusions of 10, 20 ,40 and 80 mg over 10 min to healthy volunteers demonstrated dose dependent pharmacokinetics. Plasma half life ranged between 1.5 h to 1.7 h at the higher two dose levels.

In study DX-88-1 the safety data (relevant to dose-finding) showed that 6 events of abnormal laboratory values occurred at both the 40 mg and 80 mg doses of DX-88.

Mild prolongations in aPTT were noted in 2 subjects following 40 mg DX-88 which although considered to be clinically significant, were not of a sufficient severity to warrant discontinuation of dose escalation to 80mg.

The occurrence of Grade 2 abnormal coagulation parameters (aPTT being one) following the 80 mg dose of DX-88 was of sufficient severity and number to warrant discontinuation of dose escalation according to the "stopping rules" and the planned 160mg dose was not given.

Prolongations in aPTT were all noted at the 1 hour post dose timepoint only and all had reverted to the baseline (pre dose) values by the 4 hour timepoint, indicating that these changes were transient.

Study DX-88/13

Study DX-88/13 was a Phase 1, single-center, open-label, crossover study designed to assess and compare the safety and pharmacokinetic profiles of ecallantide in healthy subjects. Subjects were administered 3 doses of ecallantide at one-week intervals: a 9.1 mg SC injection, a 27.3 mg SC

injection, or a 27.3 mg IV infusion given over 10 minutes (Days 1, 7, 14). Eighteen subjects were randomized and enrolled. Sixteen subjects received all 3 doses.

Pharmacokinetic parameters were calculated for all evaluable subjects.

Following SC administration, both mean C_{max} and AUC_{inf} increased with dose in a dose-proportional manner. The mean C_{max} was 179 ng/mL in the 9.1 mg dose group and 586 ng/mL in the 27.3 mg dose group. The mean $AUC_{0\text{-}inf}$ values were 837 ng*hr/mL and 3017 ng*hr/mL in the 9.1 mg and 27.3 mg dose groups, respectively. The t_{max} and elimination $t_{1/2}$ were similar for both SC dose groups and were approximately 2 hours.

Following IV administration, the mean C_{max} was approximately 6-fold greater than was observed in the equivalent 27.3 mg SC dose group; (mean C max [IV = 3741 ng/ml] and [SC = 586 ng/ml]) although the mean AUC0-inf values were similar between the IV and SC groups. The t1/2 of 1.6 hours following IV administration was similar to that observed following SC administration. A comparison of the mean IV and SC AUC0-inf values indicated that the absolute bioavailability of the 27.3 mg SC dose was approximately 91%.

Mean PK parameters following IV and SC administration of 30 mg (actual 27.3 mg) DX-88

are summarized in Table 5.

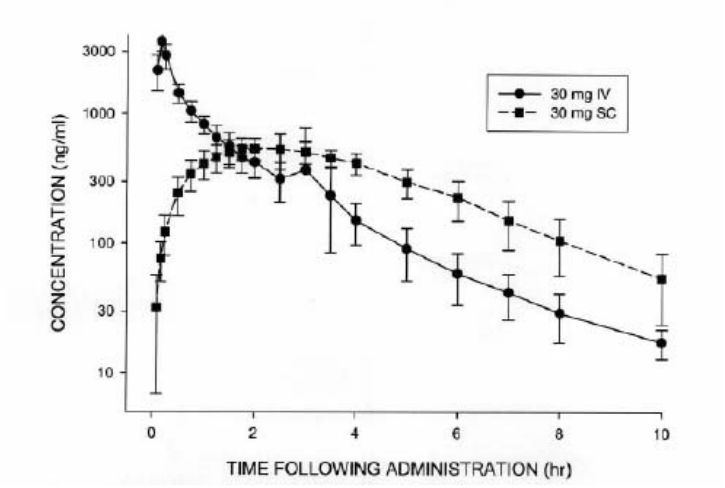
Table 5. Key Pharmacokinetic Parameters after Intravenous and Subcutaneous Administration of 30 mg (actual 27.3 mg) DX-88

Parameter	Intravenous mean ± SD	Subcutaneous mean ± SD
$t_{1/2}$ (hr)	1.60 ± 0.62	2.00 ± 0.53
Cl (mL/min)	141 ± 28.0	153 ± 20.3
Vd (L)	18.8 ± 4.55	26.4 ± 7.79
C_{max} (ng/mL)	3741 ± 460	586 ± 106*
T_{max} (hr)	0.17 ± 0.04	2.70 ± 0.65*
$AUC_{0\text{-}INF}$ (ng*hr/mL)	3327 ± 562	3017 ± 402
* Indicates p<0.05 compared to intravenous administration		

Values represent mean ± SD; administered dose was 30 mg (actual 27.3 mg) for each route of administration.
Source: Table 1, Final [PK] Report Amendment I [15] (Appendix 16.1.12).

Other significant differences with SC dosing included an increase in Tmax (mean Tmax [IV= 0.17 hr] and [SC= 2.70 hr]), and an increase in the apparent volume of distribution (mean Vd [IV= 18.8 L] and [SC= 26.4 L]).

Figure 1. Mean DX-88 Concentrations after Intravenous or Subcutaneous Administration



Values represent mean ± SD; administered dose = 30 mg (actual 27.3 mg); n = 17 for IV arm; n = 16 for SC arm.
Source: Figure 1, Final [PK] Report Amendment I [15] (Appendix 16.1.12).

Mean DX-88 concentrations after SC administration of DX-88 in the arm, abdomen, or thigh were similar. Calculated values for DX-88 elimination half-life, clearance, AUC0-INF, volume of distribution, C_{max} and T_{max} were comparable across all SC injection sites.

The maximal DX-88 concentrations were 6 times greater in the IV than the SC group and Tmax was extended from 10 minutes following IV administration to approximately 3 hours following SC administration. From the experience with other kallikrein inhibitors (e.g. aprotinin) it is known that inhibition of kallikrein seems to be correlated clinically with the C_{max}, and AUC is of minor importance. Therefore, e.g. aprotinin is only administered intravenously. The applicant is required to further justify that the difference observed regarding C_{max} and T_{max} between the IV and SC routes is not clinically relevant in the indication intended

Repeat dose PK study: DX-88-6

DX-88-6 was an open label study to assess the PK profile and safety of repeated dosing in healthy volunteers given 4 intravenous dosing regimens of DX-88 (primary objective).

The secondary objective was to assess the evidence of the levels of IgG serum antibody formation to DX-88.

Four doses of 20 mg/m2 DX-88 (ecallantide) were administered in this study. Subjects were to be dosed with 20 mg/m2 DX-88 (ecallantide) (calculated on BSA) intravenously on a weekly basis at Visit 2/Day 0 (Dose 1), Visit 3/Day 7 (Dose 2), Visit 4/Day 14 (Dose 3), and Visit 5/Day 21 (Dose 4).

Table 6. Dose Regimens

Dose Regimen	Volume Prepared[a]	Administration Time	Infusion rate (mL/hour)
Dose 1: 20 mg/m^2 over 10 minutes	20 mL	10 minutes	120 mL/hour
Dose 2: 20 mg/m^2 over 10 minutes	20 mL	10 minutes	120 mL/hour
Dose 3: 20 mg/m^2 over 10 minutes	20 mL	10 minutes	120 mL/hour
Dose 4: 20 mg/m^2 over 4 hours	480 mL	4 hours	120 mL/hour

a. *Volume prepared* refers to the total volume of DX-88 (ecallantide) and phosphate-buffered saline (PBS).

Table 7. Summary of Pharmacokinetic (PK) Parameters (Mean ± SD) from Compartmental Models of Plasma Samples Collected from Healthy Volunteers after 10-Minute or 4-Hour Intravenous Infusion of DX-88

	10-minute Infusion			4-h Infusion
	Dose 1	Dose 2	Dose 3	Dose 4
Parameter	(N=6)	(N=6)	(N=6)	(N=6)
Dose (mg)	36.5 ± 6.02	36.5 ± 6.02	36.5 ± 6.02	36.5 ± 6.02
AUC (h*µg/mL)	5.06 ± 0.94	5.75 ± 1.26	6.86 ± 2.90	5.25 ± 0.92
Half-life				
Alpha $t_{1/2}$ (h)	0.10 ± 0.04	0.13 ± 0.07	0.13 ± 0.04	0.03 ± 0.01[a]
Beta $t_{1/2}$ (h)	1.18 ± 0.44	1.89 ± 0.80	2.78 ± 2.26	1.27 ± 0.52[b]
C_{max} (µg/mL)	7.02 ± 1.36	6.47 ± 0.67	6.00 ± 0.88	1.17 ± 0.12
CL (mL/h)	7405 ± 1853	6597 ± 1849	5750 ± 1276	7101 ± 1615
Vdss (mL)	9731 ± 3152	13056 ± 3326	16770 ± 9989	11152 ± 1743

Source: Appendix 16.1.13.1 (Tables 3, 4, 5, 6)

Note: AUC = area under the curve, C_{max} = maximum plasma concentration achieved; CL = clearance; SD = standard deviation; Vdss = volume of distribution at steady state

a. (n =2) Plasma concentration data for Subjects 001-0002 and 001-0004 were consistent with a 2-compartment model, whereas data from the other 4 subjects (Subjects 001-0003, 001-0006, 001-0007, and 001-0011) were consistent with a 1-compartment model.

b: This includes terminal half-life estimated from both 1-compartment (Subjects 001-0002 and 001-0004) or 2 compartment (Subjects 001-0003, 001-0006, 001-0007, and 001-0011) modelling.

The mean plasma concentrations over time for Doses 1 to 3 (20 mg/m2 over 10 minutes) were comparable, whereas the Dose 4 infusion (20 mg/m2 over 4 hours) differed in profile compared to Doses 1 to 3.

The mean plasma concentration for Doses 1 to 3 immediately increased after DX-88 (ecallantide) administration, reaching a peak concentration at 15 minutes of just under 6 µg/mL and rapidly decreased with a concentration at 30 minutes of just over 2 µg/mL and to under 1 µg/mL at 2 hours. The Dose 4 mean plasma concentration slowly increased over time and continued to rise to a peak

concentration of just over 1 µg/mL at 4 hours and mean plasma concentration levels decreased to approximately 0.5 µg/mL at 5.5 hours.

A population PK model was developed and summarized below:

The objective of this analysis was to develop a population pharmacokinetic model for DX-88 in 62 healthy subjects, 33 patients with HAE, and 2 patients with acquired angioedema (AAE). The study designs of 7 clinical studies (4 in healthy subjects and 3 in angioedema patients) are described in this section and summarized in Table 8.

Table 8. Summary of Clinical Studies Used in the Analysis

Study	Design	Doses (number of subjects who received the dose)	Route	Subject	Sampling times	Assay (Laboratory) Assay range
DX-88/1	DB, SD ascending	10 mg (n=2) 20 mg (n=2) 40 mg (n=4) 80 mg (n=4)	IV	Healthy	Pre-dose, 5 min, 10 min (end of infusion), 15 min, 30 min, 45 min, 1, 2, 4, 6, 8, 12, and 24 hours	LC-MS (BAY) 0.5 to 20 mg/L
DX-88/2	Open-label, SD ascending	10 mg (n=3) 40 mg (n=3) 80 mg (n=3)	IV	Angioedema (HAE or AAE)	Pre-dose, 5 min, 10 min, 15 min, 30 min, 1, 2, 4, 6, 8, 12 and 24 hours	LC-MS/MS (MDS) 0.473 to 39.4 mg/L
DX-88/4	DB, SD ascending	5 mg/m^2 (n=10) 10 mg/m^2 (n=10) 20 mg/m^2 (n=10) 40 mg/m^2 (n=11)	IV	Acute attacks of HAE	Sparse sampling with 3 to 4 samples per patient after dosing	LC-MS/MS (MDS) 0.473 to 39.4 mg/L
DX-88/5	Open-label MD	5 mg/m^2 (n = 18)[a] 10 mg/m^2 (n = 55)[a] 20 mg/m^2 (n = 9)[a] 30 mg/m^2 (n = 31)[a]	IV or SC	HAE	0, 1, 2, and 4 hours after first dose	ELISA (TGA) 0.156 to 10.0 mg/L
DX-88/6	Open-label MD	20 mg/m^2 (n=8)	IV Infusions: 10 min (doses 1-3) or 4 h (4th dose)	Healthy	0 to 24 hours with 12 or 24 samples per subject	LC-MS/MS (MDS) 0.473 to 39.4 mg/L

Legend: SD, single dose; MD, multiple dose; LC-MS, high performance liquid chromatography with mass spectral detection; LC-MS/MS high performance liquid chromatography with tandem mass spectral detection HAE, hereditary angioedema; AAE, acquired angioedema; BE, bioequivalence study; SC, subcutaneous; IV, intravenous; BAY, Bay Bioanalytical Laboratory, Inc; MDS, MDS Pharma Services; TGA, TGA Sciences, Inc.
a: The number of patients in each dose level are not additive because patients in Study DX-88/5 could receive multiple doses at different dose levels and could be in more than one dose group.

Study	Design	Doses (number of subjects who received the dose)	Route	Subject	Sampling times	Assay (Laboratory)
DX-88/13	Open label crossover SD	30 mg IV (n=17) 30 mg SC (n=16) 10 mg SC into upper arm (n=6) 10 mg SC into thigh (n=6) 10 mg SC into abdomen (n=6)	IV or SC	Healthy	27 samples collected over 24 hours: Pre-dose, and 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240 minutes and 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours post-dose.	ELISA (TGA) 0.156 to 10.0 µg/L
DX-88/15	BE, DB, crossover	30 mg liquid vs. 30 mg lyophilized (n=24)	SC	Healthy	27 samples collected over 24 hours: Pre-dose, 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240 minutes and 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours post-dose.	ELISA (TGA) 0.156 to 10.0 µg/L

Legend: SD, single dose; MD, multiple dose; LC-MS, high performance liquid chromatography with mass spectral detection; LC-MS/MS high performance liquid chromatography with tandem mass spectral detection HAE, hereditary angioedema; AAE, acquired angioedema; BE, bioequivalence study; SC, subcutaneous; IV, intravenous; BAY, Bay Bioanalytical Laboratory, Inc; MDS, MDS Pharma Services; TGA, TGA Sciences, Inc.
a: The number of patients in each dose level are not additive because patients in Study DX-88/5 could receive multiple doses at different dose levels and could be in more than one dose group.

The PK from the trials in healthy volunteers and from HAE patients are consistent and support the applicant's conclusions showing dose-proportionality between 8 and 96 mg, and the relative bioavailability after SC administration of over 90%. DX-88 had a limited volume of distribution at steady state (~15.1L), which is consistent with distribution to the extracellular fluid, and was cleared rapidly (7.56 L/h) with a relatively short half-life after SC administration (□-half-life ~2 hours). The short terminal half-life means that administration of daily doses of DX-88 would not be expected to result in any significant plasma accumulation. Ecallantide is excreted in the urine. In view of this subjects with reduced renal function are expected to have prolonged exposure.

Pharmacodynamics

No formal PD tests were done in the clinical programme. Although aPTT could be considered as a PD marker, the relative effect on the clotting system compared with the kinin system is not clear.

The only PD readout that was available *in vivo* was an elevation of aPTT in those receiving a dose higher than 40mg IV. The number of subjects with an elevated aPTT was higher in the 80mg IV dose cohort compared with the 40mg dose cohort, leading to a stop in the planned dosing of subjects with 160mg IV. The higher C_{max} in the limited number of children raises the concern that a fixed dose will have more clinically significant effects on aPTT in younger and lighter subjects. In addition an increased incidence of post procedural haemorrhage as compared with placebo was seen in the cardiothoracic trials where higher doses were used.

Discussion on clinical pharmacology

In vitro studies were performed to assess the effect of ecallantide on the clotting factors and the results provide supportive evidence for a lack of a major effect on the clotting system and fibrinolytic system *in vitro* at the plasma concentration expected in HAE with the proposed posology in adult subjects of average weight. This may not apply to children and lower weight subjects in view of the proposed fixed dose. The question remains whether *in vitro* results reflect what occurs *in vivo*, as the turnover of the various factors *in vivo* will not be reflected *in vitro*.

Conclusions on clinical pharmacology

The PK from the trials in healthy volunteers and from HAE patients are consistent and support the applicant's conclusions regarding dose-proportionality between 8 and 96 mg, and the relative bioavailability after SC administration of over 90%. DX-88 had a limited volume of distribution at steady state (~15.1L), which is consistent with distribution to the extracellular fluid, and was cleared rapidly (7.56 L/h) with a relatively short γ-half-life (4.5 hours). The short terminal half-life, administration of daily doses of DX-88 would not be expected to result in any significant plasma accumulation.

No formal PD studies were performed which is considered a deficiency. The only PD readout that was available *in vivo* was an elevation of aPTT in those receiving a dose higher than 40mg IV. The number of subjects with an elevated aPTT was higher in the 80mg versus 40mg IV dose cohorts, leading to a stop in the planned dosing of subjects with 160mg.

The lack of a clinically significant effects on aPTT at the proposed posology of 30mg SC suggests that kallikrein inhibition is inadequate at this dose and further justification is required to support the applicant's position that this posology is adequate to block kallikrein activity in terms of bradykinin generation but insufficient to block kallikrein in terms of aPTT prolongation.

The higher C_{max} in the limited number of children raises the concern that a fixed dose will have more clinically significant effects on aPTT in younger and lighter subjects.

Clinical efficacy

Dose-response studies and main clinical studies

DX-88/4 (EDEMA1) and DX-88/5 (EDEMA2) assessed a range of doses in HAE. Because of the lack of a PD readout, PK efficacy and safety were considered when choosing the final proposed dose of 30mg SC.

The pivotal studies EDEMA3-DB and EDEMA4 were both double-blind, randomised, placebo controlled studies in HAE patients. EDEMA3-RD was an open-label continuation of EDEMA3-DB. The earlier studies EDEMA0 (IV administration), EDEMA1 (IV administration), EDEMA2 (IV and SC administration) can be regarded as being supportive.

The most important elements of the studies are summarized in table 9 below. A number of patients took part in more than one study.

Table 10: Overview of key elements of the clinical studies

	DX-88/2 EDEMA0	DX-88/4 EDEMA1	DX-88/5 EDEMA2	DX-88/14 EDEMA3-DB	DX-88/20 EDEMA4
Route of administration	IV	IV	IV or SC	SC	SC
Blinding	Open-label	Double-blind	Open-label	Double-blind	Double-blind
Number of patients	9 ecallantide	41 ecallantide 10 placebo	77 ecallantide	36 ecallantide 36 placebo	48 ecallantide 48 placebo
Time after onset of attack within which patients presented	10 hours	4 hours	4 hours	8 hours	8 hours
Attack severity / Number of attacks	**Not specified /** Single attack	At least moderately Severe/ Single attack	At least moderately severe / 240 attacks in the study population	Moderate or severe Single attack	Moderate or severe Single attack
Dose	10 mg 40 mg 80 mg	5 mg/m^2 10 mg/m^2 20 mg/m^2 40 mg/m^2 Placebo	IV 5 mg/m^2 10 mg/m^2 20 mg/m^2 SC 30 mg	30 mg Placebo	30 mg Placebo
Primary efficacy endpoint	Proportion of patients reporting onset of attack resolution by 4 hours	Proportion of patients with significant improvement in symptoms at primary location by 4 hours	Proportion of successful outcomes. Proportion of patients who have a partial response.	TOS at 4 hours	Change in MSCS at 4 hours
Duration of study participation	4 – 6 weeks after treatment	1 month following treatment	Varied: A patient could be treated for a maximum of 20 attacks and for 28±3 days per attack.	Including the third follow-up visit, up to 97 days. After the first follow-up visit continuation in the open-label part of study allowed.	Including the follow-up visit, 7 ± 2 days.

A total of 286 unique patients with HAE have received 1246 doses of ecallantide in the clinical development program. Across both phase III studies, 143 unique patients were randomized.

Main efficacy studies EDEMA3 and EDEMA 4.

There are two main efficacy trials, DX-88/14 (EDEMA3) and DX-88/20 (EDEMA4) both used patient reported outcomes (PROs) for presentation of efficacy as described below:

Two disease-specific PRO instruments were developed by the applicant specifically for use in EDEMA pivotal trials to measure attack-related symptom severity and improvement in relevant body sites where symptoms may have occurred. The 2 PRO scores are the Treatment Outcome Score (TOS), which evaluates global symptom response to treatment, and the Mean Symptom Complex Severity (MSCS) score, which is designed to evaluate global symptom severity at a point in time.

The Mean Symptom Complex Severity (MSCS) In EDEMA3-DB and EDEMA4, prior to treatment patients were asked to specifically identify and grade the severity of symptoms at each of 5 body sites, also known as symptom complexes. The 5 symptom complexes are: laryngeal, GI/stomach (abdominal), external head/neck (peripheral), genital/buttocks (peripheral), and cutaneous (peripheral). At specific times after treatment, patients were again asked to rate the severity of each symptom (using the definitions in Table 11), identify new or emerging symptoms at additional body sites, and assess response to treatment. These assessments were used to generate the MSCS score and the TOS (Vernon et al 2009). The MSCS is calculated by taking the arithmetic mean of the individual symptom complex severity assessments and has a possible range of 0 to 3 (Table 10). The change in MSCS is calculated as the 4-hour or 24-hour MSCS score minus the baseline MSCS score:

$$\text{MSCS score} = \frac{\Sigma \text{ symptom complex severity assessment}}{\text{number of symptom complexes}}$$

The applicant states that higher scores indicate more severe symptoms; thus, a decrease from baseline in the MSCS score represents an improvement in symptom severity. The Minimal Important Difference (MID), a threshold score of clinical relevance, was derived by the method of triangulation (Vernon et al 2009). The MID is proposed to be indicated by a reduction in MSCS of ≥0.30.

Table 10. MSCS Scoring at Baseline and Key Time Points (4 Hours and 24 Hours)

Symptom Rating	Value	Description
Normal	0	No symptoms at a particular location
Mild	1	Symptoms were noticeable but did not affect patient daily activities
Moderate	2	Symptoms affected patient daily activities and would normally cause a patient to seek treatment from a physician
Severe	3	Symptoms prevented daily activities and required treatment by a physician

At baseline, 4, and 24 hours, patients rated the severity on a categorical scale (0 = normal, 1= mild, 2 = moderate, 3 = severe) for symptoms at each affected anatomical location. Ratings are averaged to obtain the MSCS score.

This score is considered complex and while possibly acceptable as a secondary endpoint in trials of HAE where the course of the patient following treatment is not complicated by relapse, exacerbation, additional attacks of HAE at other sites or allergic reaction. However following treatment with ecallantide there are serious concerns regarding using such an integrated and complex PRO in view of the results (see below).

The MSCS readout of efficacy is considered problematic, as emerging symptom complexes that present after treatment, or worsening of secondary symptom complexes would only contribute to the MSCS in a quantitative way; this is considered a basic flaw in the MSCS as these events reflect treatment failure. In addition events such as transient worsening of the HAE may be confused in some cases with

an allergic or allergic-type reaction. These serious concerns about the design of the MSCS make it unsuitable for demonstration of efficacy.

Treatment Outcome Score (TOS)

The TOS is a composite measure of symptoms and response to treatment, weighted by severity. Three components compose the instrument: 1) identification of the symptom complex; 2) assessment of severity of each symptom at baseline; and 3) assessment of response to treatment at 4 hours and 24 hours post-treatment. At baseline, patients rank each symptom within the symptom complex.

After treatment, patients rank changes in the symptom complexes relative to baseline as shown in table 11.

Table 11. Symptom Complex Outcome Scoring at Key Time Points (4 Hours and 24 Hours)

Symptom Complex Outcome at 4 Hours and 24 Hours		
Symptom Rating	**Value**	**Description**
Significant Improvement	100	Symptom complex is "a lot better or resolved"
Improvement	50	Symptom complex is " a little better"
Same	0	Symptom complex is unchanged
Worsening	-50	Symptom complex is "a little worse"
Significant Worsening	-100	Symptom complex is "a lot worse"

The TOS is then calculated as shown in the formula below (Vernon et al 2009):

$$TOS = \frac{\sum symptom\ complex\ score \times symptom\ complex\ weight}{symptom\ complex\ weight}$$

The applicant states that higher scores indicate symptom improvement and that the MID is indicated by a TOS of ≥30 (Vernon et al 2009).

At 4 and 24 hours, patient's assessment of response were collected and recorded on a categorical scale (significant improvement [100], improvement [50], same [0], worsening [-50], significant worsening [-100]) for each symptom complex. The response to each symptom complex was weighted by the baseline severity and then averaged for the TOS.

For the TOS, severity was determined by the baseline severity of those symptom complexes present at the time of dosing **or** by the peak severity of emerging symptom complexes for those who developed new symptom complexes after treatment. Using the peak severity from an emerging symptom complex that develops after treatment for the initial presenting symptom is not endorsed. In subjects who develop a new symptom complex after treatment, this highest peak severity is used for purposes of calculating the TOS.

As stated above in the assessment of the design of the MSCS, the same concerns relate to the TOS – a patient with an emerging symptom complex is a treatment failure and the incorporation of such an event into a quantitative PRO is not acceptable.

With the MSCS and TOS designs, these allow a patient with a HAE attack to develop a new symptom after treatment – or worsening of a co-existing symptom complex other than the primary complex, and if this worsening is transient the PRO results can still be favourable at 4 hours.

As the main aim of treatment of a HAE attack is the rapid onset of relief of symptoms, these PROs are considered to complicate the interpretation of the results. In the Day 120 responses the applicant provided information on the number of cases where the severity of the TOS was based on the peak severity of an emerging symptom complex rather than the severity of the presenting symptom at baseline. There were 3 cases in the ecallantide group where emerging symptom complexes occurred after treatment in the two pivotal trials.

In the day 120 responses additional clarification was provided on how emerging symptom complexes can impact on the PRO scores (Table 12).

Table 12. Examples of How Emerging Symptom Complexes Can Be Utilized in the Calculation of MSCS Scores and TOS Values

		Symptom Complex				
		A	B	Emerging	Without ES[a]	With ES[b]
Case 1	Baseline	severe	moderate	--	2.50	2.50
	<4 hrs	--	--	moderate		
	4 hrs	mild	mild	severe	1.00	1.67
	TOS weight	100	50	-50		
	MSCS				-1.50	-0.83
	TOS				400/5=80	300/7=43
In this example the patient was showing a good response, as reflected by change in MSCS score of -1.50 and a TOS of 80. The emerging symptom complex that worsened in severity dampens the score for both TOS and change in MSCS score.						
		Symptom Complex				
		A	B	Emerging	Without ES[a]	With ES[b]
Case 2	Baseline	severe	moderate	--	2.50	2.50
	<4 hrs	--	--	moderate		
	4 hrs	mild	mild	normal	1.00	0.67
	TOS weight	100	50	100		
	MSCS				-1.50	-1.83
	TOS				400/5=80	600/7=86
This example is the same as Case 1 except that the severity of the emerging symptom complex improved rather than worsened. The scores that incorporate the outcome of the emerging symptom reflect the additional improvement.						

ES=emerging symptom complex

[a] Without accounting for the emerging symptoms, only symptom complexes A and B would be included in the calculation of MSCS and TOS.

[b] Accounting for the emerging symptom(s), all symptom complexes would be included in the calculation of TOS and the 4-hour MSCS. Baseline MSCS is the same either way if a severity of 0 is not imputed at baseline for the emerging symptom; the severity at presentation is used for determining TOS and the severity at 4 hours is used for the MSCS score.

The applicant's position that emerging symptom complexes are incorporated into the PROs is demonstrated in the examples given above. The importance of emerging symptom complexes should be considered to be such that any case with emerging symptom complexes or worsening of existing symptom complexes should be considered as a **treatment failure**, rather than have this event be reflected within a composite PRO result, as shown in table12. It is notable that the Case 1 who at 4 hours had a severe emerging symptom complex would still be counted using the MSCS as achieving the MID (probably based on a moderate symptom complex (B) which improved to mild at 4 hrs). This

example highlights the problem with these complex PROs and the inclusion of such PRO results will be expected to contribute to the overall average PRO scores for the active group in a positive way.

Interestingly for case 2, the development of an emerging symptom complex **actually improves** the MSCS score and the TOS score.

These examples highlight the difficulty and lack of clarity of these PROs in the setting of a treatment where there are problems with emerging symptom complexes (see MO31) and allergic reactions. The CHMP therefore maintains that the primary outcomes utilising such PROs are not providing clear information on efficacy and as such it is the time to response that will be the main focus of efficacy with ecallantide, acknowledging that time to onset of significant improvement in overall response and time to onset of sustained improvement were not the primary endpoints used.

The applicant was advised by CHMP Protocol Assistance that the most clinically relevant endpoint for the treatment of an acute HAE attack is time to response (time to onset of improvement, time to significant improvement and time to resolution) of the presenting HAE attack.

As a result the problems associated with the PROs used, the assessment of efficacy will focus on "time to response" parameters.

Study DX-88/14 EDEMA3

This was a Double-Blind, Placebo-Controlled Study Followed By a Repeat Dosing Phase (open label) to Assess the Efficacy and Safety of DX-88 in HAE

Approximately 35% of subjects who completed EDEMA3 went on to enrol in EDEMA 4.

Inclusion criteria

1. Age 10 years or older.
2. Documented diagnosis of HAE, Type I or II, where diagnosis met the following criteria:
   - Documented clinical history consistent with HAE
   - Documented reduction of either functional or antigenic C1-INH (below the lower limit of normal as defined by the laboratory performing the test)
   - Documented reduction of C4 (below the lower limit of normal as defined by the laboratory performing the test) and
   - Age of reported onset of first symptoms ≤25 years OR documented C1q level at or above the lower limit of normal (as defined by the laboratory performing the test).
3. Executed informed consent.
4. Enrollment Visit: An acute attack of HAE having at least one symptom complex with a severity assessment of moderate or severe.
5. Enrollment Visit: Presentation for treatment at the site within 8 hours of patient recognition of moderate-to-severe symptoms in an acute attack of HAE.

Exclusion Criteria

1. Receipt of an investigational drug or device, other than ecallantide, within 30 days prior to study enrollment.
2. Treatment with non-investigational C1-INH within 7 days prior to study enrollment.
3. Diagnosis of acquired angioedema (AAE) estrogen dependent angioedema and/or drug induced angioedema (including angiotensin-converting enzyme inhibitor induced angioedema).
4. Pregnancy or breast feeding.

5. Patients who received ecallantide in EDEMA2 or any other studies with ecallantide within 7 days of presentation for dosing in the double-blind part.

Objectives
Primary Efficacy Outcome Measure

- Treatment Outcome Score (TOS) at 4 hours based on the severity assessment of symptom complex(es) as determined by the patient

Secondary Efficacy Outcome Measures

- Change in Mean Symptom Complex Severity (MSCS) at 4 hours
- Time to onset of significant improvement in overall response

The time in minutes to report the first significant improvement in overall response was defined as the first time (in minutes) post-dosing that the patient reported the overall assessment as "a lot better or resolved."

Of the 2 secondary endpoints in EDEMA3 the Time to significant improvement in overall response is considered the most relevant.

Tertiary Efficacy Outcome Measures

- Durability of response
- TOS at 4 hours based on the severity assessment of symptom complex(es) as determined by the Investigator
- Proportion of responders at 4 hours

  (A successful response was defined as TOS at 4 hours post-dosing based on patient's severity assessment of greater than or equal to 70)
- Time to onset of sustained improvement

  (A sustained improvement in overall response was the patient reporting feeling "a little better," "a lot better or resolved" on the eDiary) for a continuous duration of greater than or equal to 45 minutes)
- Proportion of patients receiving medical intervention
- Assessment of open-label treatment with ecallantide for severe upper airway compromise (SUAC)
- Change in clinical laboratory measures

Of the 7 tertiary endpoints in EDEMA3 the time to onset of sustained improvement in overall response is considered the most relevant.

Safety Outcome Measures

- • Adverse events
- • Laboratory test results
- • Electrocardiogram (ECG) findings
- • Antibodies to ecallantide and Pichia pastoris
- • Vital signs

Randomisation and treatment
In the double-blind part, 72 patients were randomized 1:1 to ecallantide or placebo. Randomization followed a block design, stratified according to prior use of ecallantide and attack location. Individual patients were treated for only one acute attack of HAE in the double blind part of the study.

Study drug was administered as a single dose by three 1 mL SC injections of either ecallantide (30 mg total) or placebo administered in the thigh, abdomen, and/or upper arm.

After initial dosing with study drug in the double-blind part of the study, standard of care (eg, C1-INH, where available; fresh frozen plasma; high dose androgens) or a single open-label dose of 30 mg ecallantide could be administered to the patient if the Investigator judged that the patient was at risk of severe upper airway compromise (SUAC).

Patients who received additional HAE therapy within 4 hours of treatment with study drug were censored at the time of the medical intervention in time-to-event analyses

Patient disposition

Table 13 summarizes the disposition of patients in the EDEMA3 study. Equal numbers of patients were included for analysis in the ITT-as-randomized, ITT-as-treated, and Safety Populations. Patient 361004 in the ecallantide treatment group was omitted from analysis in the Per Protocol population because he failed to complete his baseline and 4-hour post-dose assessment due to an eDiary malfunction. Seventy-one of 72 patients (98.6%) completed the study; 1 patient in the ecallantide group was lost to follow-up after Visit 1 (Patient 317006). No patients withdrew due to AEs.

During the conduct of the study, 2 patients were randomized on the same day at the same study center and administered the incorrect treatment. One patient randomized to the ecallantide treatment group received placebo and 1 patient randomized to the placebo group received ecallantide. Therefore, the ITT population was analyzed both according to the population as randomized (ITT-as-randomized) and the population as treated (ie, ITT-as-treated).

EDEMA 3 Table 13 Summary of Patient Disposition

	DX-88 (N=36)	Placebo (N=36)	Total (N=72)
	n (%)	n (%)	n (%)
Intent to Treat Population as Randomized[a]	36 (100.0)	36 (100.0)	72 (100.0)
Intent to Treat Population as Treated[b]	36 (100.0)	36 (100.0)	72 (100.0)
Per Protocol Population[c]	35 (97.2)	36 (100.0)	71 (98.6)
Safety Population[d]	36 (100.0)	36 (100.0)	72 (100.0)
Patients completing Study (double-blind)	35 (97.2)	36 (100.0)	71 (98.6)
Patients withdrawing from Study	1 (2.8)	0	1 (1.4)
Patients continuing onto Open-Label Part	21 (58.3)	27 (75.0)	48 (66.7)
If withdrawing from study, Primary Reason			
Adverse Event	0	0	0
Protocol Violation	0	0	0
Did not complete dosing	0	0	0
Did not complete 4 hour follow-up assessment	0	0	0
Lost to follow-up	1 (2.8)	0	1 (1.4)

Source: Summary Table 14.1.1

[a] Patients who received any amount of study drug and who completed their 4 hour follow-up assessment, analyzed with treatment assigned.

[b] Patients who received any amount of study drug and who completed their 4 hour follow-up assessment, analyzed with treatment actually received.

[c] Patients who received a completed dose of study drug and completed their 4 hour follow-up assessment with no major protocol violations.

[d] Patients who received any amount of study drug.

Baseline demographics and disease characteristics

Table 14 Summary of Demographics (ITT-as-Randomized, ITT-as-Treated, and Per Protocol Populations)

Parameter	Statistic	Population: ITT-as-randomized			Population: ITT-as-treated			Population: Per Protocol		
		DX-88 (N=36)	Placebo (N=36)	Total (N=72)	DX-88 (N=36)	Placebo (N=36)	Total (N=72)	DX-88 (N=35)	Placebo (N=36)	Total (N=71)
Age	N	36	36	72	36	36	72	35	36	71
	Mean	38.5	32.2	35.4	37.1	33.6	35.4	37.4	33.6	35.5
	Median	37.4	30.4	33.7	36.9	31.3	33.7	37.3	31.3	34.4
	Std. Dev.	14.60	13.81	14.46	14.27	14.64	14.46	14.38	14.64	14.54
	Range (min,max)	(18,77)	(11,57)	(11,77)	(16,77)	(11,66)	(11,77)	(16,77)	(11,66)	(11,77)
Gender										
Male	n (%)	12 (33.3)	13 (36.1)	25 (34.7)	13 (36.1)	12 (33.3)	25 (34.7)	12 (34.3)	12 (33.3)	24 (33.8)
Female	n (%)	24 (66.7)	23 (63.9)	47 (65.3)	23 (63.9)	24 (66.7)	47 (65.3)	23 (65.7)	24 (66.7)	47 (66.2)
Race										
White	n (%)	33 (91.7)	32 (88.9)	65 (90.3)	33 (91.7)	32 (88.9)	65 (90.3)	32 (91.4)	32 (88.9)	64 (90.1)
Black	n (%)	1 (2.8)	4 (11.1)	5 (6.9)	1 (2.8)	4 (11.1)	5 (6.9)	1 (2.9)	4 (11.1)	5 (7.0)
Hispanic	n (%)	2 (5.6)	0	2 (2.8)	2 (5.6)	0	2 (2.8)	2 (5.7)	0	2 (2.8)

Source: Summary Table 14.1.5.1, Summary Table 14.1.5.2, and Summary Table 14.1.5.3

The only difference between the ITT as planned and the ITT-as treated is a one patient in each group who received the incorrect treatment in the ITT-as-planned.

HAE Attack History

Table 15 HAE Attack History at Baseline (ITT-as-Randomized Population and ITT-as-Treated Population)

Parameter	Statistic	ITT-as-randomized		ITT-as-treated	
		DX-88 (N=36)	Placebo (N=36)	DX-88 (N=36)	Placebo (N=36)
Age at First HAE Symptoms Onset (yrs)	N	36	36	36	36
	Mean	12.1	10.3	11.7	10.8
	Median	12.0	9.5	11.5	10.0
	Std. Dev.	6.50	6.86	6.70	6.75
	(Min, Max)	(1, 32)	(1, 25)	(1, 32)	(1, 25)
Lowest Historical Functional C1-INH Level (%)	N	29	25	29	25
	Mean	18.7	22.8	20.0	21.3
	Median	13.0	12.0	17.9	11.9
	Std. Dev.	20.37	24.79	20.39	24.94
	(Min, Max)	(0, 59)	(0, 97)	(0, 59)	(0, 97)
Lowest Historical Antigenic C1-INH Level (mg/dL)	N	26	27	26	27
	Mean	22.4	18.4	23.2	17.6
	Median	11.1	9.0	11.1	9.0
	Std. Dev.	24.00	21.83	24.62	20.98
	(Min, Max)	(3, 79)	(0, 80)	(3, 79)	(0, 80)
Lowest Historical C4 Level	N	34	34	34	34

Parameter	Statistic	ITT-as-randomized		ITT-as-treated	
		DX-88 (N=36)	Placebo (N=36)	DX-88 (N=36)	Placebo (N=36)
(mg/dL)	Mean	10.6	9.9	9.2	11.2
	Median	6.0	5.3	6.0	5.3
	Std. Dev.	12.87	13.54	10.20	15.60
	(Min, Max)	(0, 55)	(0, 56)	(0, 55)	(0, 56)

Source: Summary Table 14.1.9.1.1 and Summary Table 14.1.9.1.2

The lowest historical functional C1INH is listed as up to 97% of normal, but the applicant response at day 120 stated that this patient had a clinical diagnosis of HAE and low C1INH antigen levels and low C4. Therefore while these results are not fully consistent for a diagnosis of HAE, as this is only a single case, with other features of HAE, the response can be accepted.

Prior HAE Treatment: Prophylaxis

In the ITT-as-randomized population, danazol or stanozolol were used prophylactically by 58.3% and 47.2%, respectively, of patients in the ecallantide treatment group, compared to 38.9% and 33.3%, respectively for patients in the placebo group.

More patients in the active arm were on treatment with Danazol, Stanozolol and aminocaproic acid. As prior history of prophylaxis was taken at enrolments and not again at baseline – this leads to a possible bias in favour of the active group. Further information on time to response endpoints for those with and without prophylactic medications has been requested.

Baseline Characteristics for Treated Attacks

Table 16 Symptom Complex Identification at Baseline - Patient Report (ITT-as-Randomized, ITT-as-Treated, and Per Protocol Populations

Symptom Complex	Severity	Statistic	Population: ITT-as-randomized		Population: ITT-as-treated		Population: Per Protocol	
			DX-88 (N=36)	Placebo (N=36)	DX-88 (N=36)	Placebo (N=36)	DX-88 (N=35)	Placebo (N=36)
Internal Head/ Neck Symptoms	Mild	n (%)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.9)	1 (2.8)
	Moderate	n (%)	7 (19.4)	1 (2.8)	7 (19.4)	1 (2.8)	6 (17.1)	1 (2.8)
	Severe	n (%)	1 (2.8)	2 (5.6)	1 (2.8)	2 (5.6)	1 (2.9)	2 (5.6)
Stomach/ Gastrointestinal	Mild	n (%)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.9)	1 (2.8)
	Moderate	n (%)	14 (38.9)	13 (36.1)	13 (36.1)	14 (38.9)	13 (37.1)	14 (38.9)
	Severe	n (%)	5 (13.9)	7 (19.4)	5 (13.9)	7 (19.4)	5 (14.3)	7 (19.4)
Genital/Buttocks	Mild	n (%)	0	0	0	0	0	0
	Moderate	n (%)	1 (2.8)	3 (8.3)	1 (2.8)	3 (8.3)	1 (2.9)	3 (8.3)
	Severe	n (%)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.8)	1 (2.9)	1 (2.8)
External Head/ Neck	Mild	n (%)	2 (5.6)	3 (8.3)	2 (5.6)	3 (8.3)	2 (5.7)	3 (8.3)
	Moderate	n (%)	1 (2.8)	3 (8.3)	1 (2.8)	3 (8.3)	1 (2.9)	3 (8.3)
	Severe	n (%)	1 (2.8)	3 (8.3)	2 (5.6)	2 (5.6)	2 (5.7)	2 (5.6)
Cutaneous	Mild	n (%)	4 (11.1)	1 (2.8)	4 (11.1)	1 (2.8)	4 (11.4)	1 (2.8)
	Moderate	n (%)	13 (36.1)	11 (30.6)	13 (36.1)	11 (30.6)	13 (37.1)	11 (30.6)
	Severe	n (%)	4 (11.1)	2 (5.6)	4 (11.1)	2 (5.6)	4 (11.4)	2 (5.6)

Source: Modifications to Summary Tables 14.1.13.1.1, 14.1.13.1.2, and 14.1.13.1.3

Note: Symptom Complex Identification taken from Patient pre-dose eDiary.

Note: A patient may have more than 1 Symptom Complex present.

Multiple symptom complexes:

The percentage of cases with one or more symptom complexes at presentation is provided in the table 17.

Table 17 Symptom Complexes and Emerging Symptoms in EDEMA3-DB

	EDEMA3-DB	
# symptom complexes at Baseline	DX-88 N=36 n (%)	Placebo N=36 n (%)
1	19 (52.8)	26 (72.2)
2	16 (44.4)	5 (13.9)
3	0 (--)	4 (11.1)
4	1 (2.8)	1 (2.8)
5	0 (--)	0 (--)
# patients with emerging symptom complexes at up to 4 hours post treatment	1 (2.8)	3 (8.3)

It is noted that far more subjects in the ecallantide arm (47.2%) had more than one symptom complex at baseline than the placebo group (27.8%) in EDEMA 3.

Outcomes and estimation

Primary outcome

Treatment Outcome Score (TOS) at 4 hours based on the severity assessment of symptom complex(es) as determined by the patient

Table 18. Primary Efficacy Analysis: Treatment Outcome Score (TOS) at 4 Hours Post-Dosing (ITT-as-Randomized, ITT-as-Treated, and Per Protocol Populations)

	Population: ITT-as-randomized			Population: ITT-as-treated			Population: Per Protocol		
Statistic	DX-88 (N=36)	Placebo (N=36)	*P*-value[a]	DX-88 (N=36)	Placebo (N=36)	*P*-value[a]	DX-88 (N=35)	Placebo (N=36)	*P*-value[a]
N	36	36	0.100	36	36	0.037*	35	36	0.021*
Mean	46.8	21.3		49.5	18.5		53.8	18.5	
Median	50.0	0.0		50.0	0.0		50.0	0.0	
Std. Dev.	59.34	69.04		59.43	67.78		54.40	67.78	
(Min, Max)	(-100,100)	(-100,100)		(-100,100)	(-100,100)		(-100,100)	(-100,100)	
(25th, 75th)	(0, 100)	(0, 100)		(0, 100)	(0, 100)		(0, 100)	(0, 100)	

Sources: Summary Tables 14.2.1.1.1, 14.2.1.2.1, and 14.2.1.3.1

[a] *P*-value from Wilcoxon Rank Sum Test. *Statistically significant result ($P<0.05$).

This outcome measure is considered problematic and is not the most clinically relevant outcome for treatment of an acute attack of HAE, which is the time to response.

Secondary outcomes

Change in MSCS from Baseline

Table 19 Mean Symptom Complex Score (MSCS): Change from Baseline in MSCS at 4 Hours Post-Dosing (ITT-as-Randomized and ITT-as-Treated Populations)

	ITT-as-randomized Population						ITT-as-treated Population					
	Baseline		4h Post-Dose		Change from BL		baseline		4h Post-Dose		Change from BL	
Statistic	DX (N=36)	PL (N=36)	DX (N=36)	PL (N=36)	DX (N=36)	PL (N=36)	DX (N=36)	PL (N=36)	DX (N=36)	PL (N=36)	DX (N=36)	PL (N=36)
Mean	2.15	2.26	1.26	1.75	-0.88	-0.51	2.17	2.24	1.26	1.75	-0.91	-0.48
Median	2.00	2.00	1.00	2.00	-1.00	-0.50	2.00	2.00	1.00	2.00	-1.00	-0.42
Std. Dev.	0.49	0.56	0.96	0.90	1.11	0.68	0.51	0.55	0.96	0.90	1.10	0.68
Min, Max	1.50, 3.00	1.25, 3.00	0.00, 3.00	0.00, 3.00	-3.00, 1.50	-1.67, 1.75	1.50, 3.00	1.25, 3.00	0.00, 3.00	0.00, 3.00	-3.00, 1.50	-1.67, 1.75
25th, 75th	2.00, 2.38	2.00, 3.00	0.50, 2.00	1.00, 2.50	-1.50, 0.00	-1.00, 0.00	2.00, 2.50	2.00, 3.00	0.50, 2.00	1.00, 2.50	-1.50, -0.25	-1.00, 0.00
P-value					0.094						0.044*	

Sources: Summary Tables 14.2.2.1.1 and 14.2.2.2.1

DX = ecallantide; PL = placebo; BL = baseline

P-value from Wilcoxon Rank Sum Test. *Statistically significant result ($P<0.05$).

Similar to the primary endpoint of TOS, this secondary endpoint of MSCS is considered problematic and is not the most clinically relevant endpoint for treatment of a HAE attack.

Time to Significant Improvement

Table 20 Time to Report of Significant Improvement in Overall Response (Kaplan-Meier) ITT-as-Randomized, ITT-as-Treated, and Per Protocol Populations

	Population: ITT-as-randomized			Population: ITT-as-treated			Population: Per Protocol		
	DX-88 (N=36)	Placebo (N=36)	*P*-value	DX-88 (N=36)	Placebo (N=36)	*P*-value	DX-88 (N=35)	Placebo (N=36)	*P*-value
Patients with Significant Improvement[a], n (%)	18 (50.0)	12 (33.3)		19 (52.8)	11 (30.6)		19 (54.3)	11 (30.6)	
Patients with censored data[b], n (%)	18 (50.0)	24 (66.7)		17 (47.2)	25 (69.4)		16 (45.7)	25 (69.4)	
Estimated Time to First Significant Improvement (minutes)[a]			0.136			0.055			0.045*
Median	165.0	--		165.0	--		149.0	--	
Interquartile Range	(83.0, --)	(135.0,--)		(83.0, --)	(135.0,--)		(83.0, --)	(135.0,--)	

Sources: Summary Tables 14.2.3.1, 14.2.3.2, and 14.2.3.3

[a] Time to report of significant improvement was defined as the first time (in minutes) post-dosing that the patient reported the overall response assessment as "a lot better or resolved."

[b] Patients receiving additional HAE therapy for SUAC within 4 hours were censored at the time of the medical intervention. Patients not reporting the overall response assessment as "a lot better or resolved" in 15 minutes through 4 hours post-dosing were censored at 240 minutes.

*Statistically significant result ($P<0.05$), log-rank test.

For the ITT-as-treated the time to report of significant improvement in overall response was not statistically significantly different from placebo. The % censored was high in both treated and placebo groups in ITT-as-treated analysis; 47% in the ecallantide group and 69% in placebo. This does not suggest adequate efficacy. As censoring was done only for those received additional treatment for SUAC with severe upper airways compromise (SUAC) within 4 hrs and for those not reporting improvement by 4 hours, efficacy was poor in both groups. The p values were not significant for the ITT-as-treated but were for the PP. The difference in the population in the PP versus the ITT as treated was the removal of one case who didn't fill in his diary at all time points.

Tertiary Efficacy Endpoints Analysis

Durability of response

For both the ITT-as-randomized and ITT-as-treated populations, data were identical. Median (IQR) TOS at 24 hours post-dose was 75.0 (0, 100) in the ecallantide treatment group compared to 0.0 (-100, 100) in the placebo group (P=0.044).

The clinical relevance of durability of response at 24 hours is questioned when the data provided does not demonstrate clear evidence of efficacy on time to significant improvement. It is rapid treatment that is required for the serious HAE attacks that is effective in the majority of patients and that is sustained. In addition the number of AEs of HAE in the ecallantide group was higher than in the placebo group at day 0 (AEs of HAE n=8 and n= 4 respectively), day 1 (n=7 for ecallantide group, not reported for placebo) and day 2 (n=5 and n=2 respectively). This data is not consistent with durability of response.

Analysis of Change from Baseline in MSCS at 24 hours Post-Dosing

For the ITT-as-randomized the median (IQR) MSCS at baseline for the ecallantide treatment group was 2.00 (2.00, 2.00) and for the placebo group was 2.00 (2.00, 3.00). At 24 hours post-dose, median (IQR) MSCS was 1.00 (0.50, 2.00), the lower range of moderate scoring of the symptom complexes. At the same time point, the placebo group MSCS (IQR) was 1.33 (1.00, 3.00), the upper range of moderate scoring. However, this changes did not reach significance (P=0.142).

Time to Onset of Sustained Improvement in Overall Response

Table 21 Time to Onset of Sustained Improvement in Overall Response (Kaplan-Meier) (ITT-as-Randomized and ITT-as-Treated Populations)

	Population: ITT-as-randomized			Population: ITT-as-treated		
	DX-88 (N=36)	Placebo (N=36)	*P*-value	DX-88 (N=36)	Placebo (N=36)	*P*-value
Patients with Sustained Improvement[a], n(%)	25 (69.4)	18 (50.0)		26 (72.2)	17 (47.2)	
Patients with Censored Data[b], n(%)	11 (30.6)	18 (50.0)		10 (27.8)	19 (52.8)	
Estimated Time to Onset of Sustained Improvement, (minutes)[a]			0.075			0.023*
Median	67.0	165.0		67.0	165.0	
Interquartile range	37.0, --	49.0, --		37.0, 135.0	49.0, --	

Sources: Summary Table 14.2.6.1 and Summary Table 14.2.6.2

[a] Time to onset of sustained improvement is defined as the first time (in minutes) throughout the first 4 hours that a patient had an improvement ('a little better or a lot better or resolved') for a continuous duration of ≥ 45 minutes.

[b] Patients not reporting sustained improvement post-dosing were censored at 240 minutes. Patients receiving additional HAE therapy for SUAC within 4 hours were censored at the time of the medical intervention.

*Statistically significant result ($P<0.05$), log-rank test.

The small numbers of those who had a sustained improvement reaches statistical significance for the ITT-as-treated. In view of the high proportion of patients in both active and placebo arms who were censored this tertiary endpoint is not considered to provide robust evidence of efficacy.

Proportion of Successful Response Assessment at 4 hours post-dosing

A successful response was defined as a TOS of ≥70 based on patient-assessment, at 4 hours post-dosing, and had been chosen based on limited experience with the TOS. In the ITT-as-randomized population, 15 of 36 (41.7%) patients treated with ecallantide and 12 of 36 (33.3%) patients who received placebo had a successful response

In the ITT-as-treated population, 16 of 35 (44.4%) patients treated with ecallantide and 11 of 36 (30.6%) patients who received placebo had a successful response.

Less than half of the treated patients had a successful response in the ecallantide treated arm. This is considered inadequate for treatment of HAE attacks.

Proportion of Patients Receiving Medical Intervention

In the ITT-as-randomized analysis population, a greater proportion of patients who received placebo (13 of 36, 36.1%) required medical intervention within 24 hours of administration of placebo compared to patients treated with ecallantide (5 of 36, 13.9%).

In both treatment groups, medical intervention typically consisted of emergency medications (eg, opioid and non-opioid analgesics for pain, anti-nausea medications, or C1-INH).

The percentage of patient requiring medical intervention did not include those requiring supportive care (eg, IV fluids). The applicant's response addressed the additional intervention of IV fluids and further clarification is requested on the censoring applied to these cases.

The overall conclusions on the efficacy data provided from EDEMA3 is that efficacy has not been robustly demonstrated for the clinically relevant endpoints, namely time to response (time to onset of improvement, time to significant improvement and time to resolution). The efficacy data provided for the primary endpoint and for the secondary endpoint of change in MSCS are not considered to be the most clinically relevant and are hampered by the complexity of interpretation as discussed above. The secondary endpoint of Time to Significant Improvement is not statistically significant for the ITT-as-treated. Furthermore when the integrated analysis of the two DB trials was combined and time to response was analysed by anatomical location, these results were inconsistent across different attack locations (see analysis performed across trials below).

DX-88/20 EDEMA4

This was a Phase 3, randomized, double-blind, placebo-controlled, multicenter study, multicenter trial designed to assess the efficacy and safety of 30 mg subcutaneous (SC) DX-88 (ecallantide) vs placebo in the treatment of moderate to severe acute attacks of hereditary angioedema (HAE).

Inclusion criteria

1. 10 years of age or older
2. Executed informed consent
3. Documented diagnosis of HAE (Type I or II), based upon:
    a. Documented clinical history consistent with HAE (SC or mucosal, nonpruritic swelling without accompanying urticaria)
    b. Functional or antigenic C1-INH level below the lower limit of the normal range or up to 15% above the lower limit of the normal range as defined by the reference laboratory
    c. C4 level below the lower limit of the normal range or up to 15% above the lower limit of the normal range as defined by the reference laboratory
    d. Age of reported onset of first angioedema symptoms ≤25 years or documented complement component 1q (C1q) level at or above the lower limit of the normal range as defined by the reference laboratory
4. Enrollment visit: Presentation at the site within 8 hours of patient recognition of an acute attack of HAE with at least one moderate to severe symptom complex
5. Men and women who were sexually active and fertile must have been practicing at least 2 methods of contraception for the duration of the study (eg, oral contraceptive plus condom, intrauterine device [IUD] plus condom, diaphragm with spermicidal cream plus condom)

Exclusion Criteria

Patients who met any of the following criteria were to be excluded from the study:

1. Receipt of an investigational drug or device within 30 days prior to study treatment, with the exception of:
   a. Treatment with C1 inhibitor concentrate (C1-INH) for angioedema within 7 days prior to study treatment
   b. Treatment with ecallantide within 3 days prior to study treatment
2. Diagnosis of acquired angioedema (AAE), estrogen-dependent angioedema, and/or drug-induced angioedema (including angiotensin-converting enzyme inhibitor-induced angioedema)
3. Pregnancy or breast feeding
4. Any other condition that, in the opinion of the Investigator, may have compromised the safety or compliance of the patient or would have precluded the patient from successful completion of the study

Of note is that the inclusion criterion 3b differs from the inclusion criteria in EDEMA3. 25 subjects in EDEMA 4 had taken part in EDEMA 3.

Objectives

The primary objective of EDEMA4 was to compare the change from baseline in the Mean Symptom Complex Severity (MSCS) score at 4 hours post-dosing between patients treated with ecallantide and those treated with placebo.

Secondary Objectives

The secondary objectives of this study were to compare ecallantide and placebo groups with regard to the following:

- Treatment Outcome Score (TOS) at 4 hours, based on the patient's severity assessment of symptom complexes at baseline
- Time to significant improvement in overall response
- Proportion of patients maintaining a significant improvement in overall response
- Proportion of responders at 4 hours, based on the change from baseline in the MSCS Score

For this endpoint, a successful response was defined as an improvement in an existing laryngeal symptom complex, stabilization of an existing peripheral symptom complex (eg, the 4-hour score for this symptom complex is no worse than at baseline), or a change in the MSCS score from baseline to 4 hours. For this analysis, the latter 2 criteria (improvement of laryngeal symptoms and no change in peripheral symptoms) were based on the overall assessment of response (ie, on the overall outcome based on all symptoms, not on the specific outcome of the laryngeal or peripheral symptoms). Stomach/GI symptoms were included as peripheral symptoms.

The definition of a responder for the endpoint Proportion of responders at 4 hours, based on the change from baseline in the MSCS Score is considered problematic. Stabilization of peripheral swelling is not considered efficacy as the patients presented relatively late in an attack and so stabilization may be the natural course of the attack. In addition the distinction between "overall outcome" and specific attack outcome is also problematic. It was noted that assessment of time to response parameters for laryngeal attacks was poorer if the laryngeal attacks only were considered compared to results displaying the time to response parameters for laryngeal attacks when "overall response" was used.

This definition of response is considered to be very weak and not sufficiently conservative.

Of the 4 secondary endpoints in EDEMA4 the Time to significant improvement in overall response

is considered the most relevant.

Tertiary Objectives

- Durability of response at 24 hours post-dosing by the change from baseline in the MSCS score and by TOS
- TOS at 4 hours post-dosing based on the Investigator's severity assessment of symptom complexes at baseline
- Proportion of responders at 4 hours post-dosing, based on TOS ≥70 and TOS ≥50
- Time to onset of sustained improvement in overall response
- Proportion of patients receiving medical intervention during an attack
- Response to open-label dosing for failed or incomplete response or for relapse, based on the change from baseline in the MSCS score at 4 hours after Dose B
- Response to open-label treatment for severe upper airway compromise (SUAC), based on the change from baseline in the MSCS score at 4 hours after SUAC dose
- Number and types of medical intervention during the 4-hour period after dosing

Of the tertiary endpoints in EDEMA4 the time to onset of sustained improvement in overall response is considered the most relevant.

Treatments and randomisation

Patients were to be randomized 1:1 to receive ecallantide or placebo.

Patient disposition

Table 22. Summary of Patient Disposition (All Populations

	Treatment Group		
	Ecallantide (N=48) n (%)	Placebo (N=48) n (%)	Total (N=96) n (%)
Intent-to-treat population[a]	48 (100.0)	48 (100.0)	96 (100.0)
Per-protocol population[b]	47 (97.9)	48 (100.0)	95 (99.0)
Safety population[c]	48 (100.0)	48 (100.0)	96 (100.0)
Patients completing study (through Follow-up Visit 1)	48 (100.0)	47 (97.9)	95 (99.0)
Patients rolling over to continuation study[d]	47 (97.9)	46 (95.8)	93 (96.9)
Patients withdrawing from study	0	1 (2.1)	1 (1.0)
Primary reason for withdrawal:			
Adverse event	0	0	0
Noncompliance or protocol violation	0	0	0
Patient withdrawal of consent	0	0	0
Lost to follow up	0	0	0
Investigator discretion	0	0	0
Termination of the study by the Sponsor	0	0	0
Other	0	1 (2.1)	1 (1.0)
Left study site against medical advice	0	1 (2.1)	1 (1.0)

Source: Summary Table 14.1.1

[a] Patients who received any amount of study drug.

[b] Patients who received a complete dose of study drug with no major protocol violations.

[c] Patients who received any amount of study drug.

[d] All patients were to be enrolled in DX-88/19, an open-label extension study, for follow-up safety assessments. A total of 2 patients (1 in the ecallantide group and 1 in the placebo group) who completed the study declined further participation and did not roll over to the continuation (extension) study (Appendix 16.2.4.16).

Demographics

Table 23 summarizes the demographic characteristics of patients in the ITT population, by treatment group and overall.

Table 23. Summary of Demographics (ITT Population)

	Treatment Group		
	Ecallantide (N=48)	Placebo (N=48)	Total (N=96)
Age, years[a]			
N	48	48	96
Mean (SD)	37.0 (13.12)	38.0 (12.19)	37.5 (12.61)
Median	34.5	38.6	37.8
(Min, max)	(15.98, 72.77)	(13.64, 72.37)	(13.64, 72.77)
Sex, n (%)			
N	48	48	96
Male	11 (22.9)	20 (41.7)	31 (32.3)
Female	37 (77.1)	28 (58.3)	65 (67.7)
Race, n (%)			
N	48	48	96
White	39 (81.3)	43 (89.6)	82 (85.4)
Black	3 (6.3)	3 (6.3)	6 (6.3)
Asian	1 (2.1)	1 (2.1)	2 (2.1)
Hispanic	4 (8.3)	1 (2.1)	5 (5.2)
Other	1 (2.1)	0	1 (1.0)

Source: Summary Table 14.1.6.1

Abbreviations: max = maximum; min = minimum; SD = standard deviation

[a] Age on the date the ICF was signed.

	Treatment Group		
Parameter	Ecallantide (N=48)	Placebo (N=48)	Total (N=96)
Age at first HAE symptoms onset, years[a]			
N	47	48	95
Mean (SD)	13.4 (7.41)	13.0 (9.46)	13.2 (8.47)
Median	13.0	13.0	13.0
(Min, max)	(0.00, 44.00)	(1.00, 59.00)	(0.00, 59.00)
Lowest historical functional C1-INH level, %[b]			
N	25	24	49
Mean (SD)	31.8 (20.10)	22.7 (19.55)	27.3 (20.15)
Median	30.0	14.0	26.0
(Min, max)	(0.11, 78.00)	(0.00, 61.00)	(0.00, 78.00)
Lowest historical antigenic C1-INH level, mg/dL[b]			
N	20	25	45
Mean (SD)	10.2 (17.05)	12.7 (23.19)	11.6 (20.50)
Median	6.2	4.0	5.0
(Min, max)	(0.00, 80.00)	(2.40, 90.00)	(0.00, 90.00)
Lowest historical C4 level, mg/dL[b]			
N	34	38	72
Mean (SD)	8.8 (13.23)	10.0 (10.88)	9.4 (11.97)
Median	5.4	7.5	6.2
(Min, max)	(0.00, 59.00)	(1.32, 60.00)	(0.00, 60.00)

Source: Summary Table 14.1.10.1

Abbreviations: C1-INH = C1 esterase inhibitor; C4 = complement component 4; HAE = hereditary angioedema; ITT = intent-to-treat; max = maximum; min = minimum; SD = standard deviation

[a] Age at HAE onset derived from (year of first HAE symptoms – year of birth)

[b] Data for laboratory values in units not convertible to the selected units were not included.

The age range at first symptom onset ranges from 0 yrs to 59yrs.

59 years is considered very late for presentation of fist HAE episode. The applicant‘s day 120 responses provided further information on the enrolled patients supporting the underlying diagnosis of HAE.

Table 25. Patients Treated in Prior Ecallantide Studies (ITT Population)

	Treatment Group		
	Ecallantide (N=48) n (%)	Placebo (N=48) n (%)	Total (N=96) n (%)
Patients treated in prior ecallantide studies			
Yes	17 (35.4)	19 (39.6)	36 (37.5)
No	31 (64.6)	29 (60.4)	60 (62.5)
Participation in:[a]			
DX-88/4 (EDEMA1)	3 (6.3)	2 (4.2)	5 (5.2)
DX-88/5 (EDEMA2)	4 (8.3)	8 (16.7)	12 (12.5)
DX-88/14 (EDEMA3)	16 (33.3)	15 (31.3)	31 (32.3)

Source: Summary Table 14.1.3

Abbreviations: ITT = intent-to-treat

[a] A patient may have participated in more than one study. Patients in EDEMA3 were from both the double-blind and repeat-dosing parts.

More than 30% of the patients in each group had received ecallantide previously. This raised statistical concerns over the possible enrichment of the study population, but on review of the sensitivity analyses provided by the applicant it was demonstrated that no enrichment for efficacy was found.

Concomitant Medications (Screening)

In contrast to EDEMA3, in EDEMA4 the two groups were matched for concomitant anabolic or attenuated androgens

Baseline Characteristics for Treated Attacks

Table 26. Patient-Reported Symptom Complex Severity at Baseline (ITT Population)

	Treatment Group		
Symptom Complex[a] Severity	Ecallantide (N=48) n (%)[b]	Placebo (N=48) n (%)[b]	Total (N=96) n (%)[b]
Patients with one or more symptom complexes at baseline	48 (100.0)	47[d] (97.9)	95 (99.0)
Number of symptoms complexes at baseline[c]	80	75	155
Internal Head/Neck Symptoms			
Mild	0	6 (12.8)	6 (6.3)
Moderate	6 (12.5)	6 (12.8)	12 (12.6)
Severe	2 (4.2)	1 (2.1)	3 (3.2)
Stomach/GI			
Mild	5 (10.4)	1 (2.1)	6 (6.3)
Moderate	9 (18.8)	20 (42.6)	29 (30.5)
Severe	4 (8.3)	6 (12.8)	10 (10.5)
Genital/Buttocks			
Mild	0	1 (2.1)	1 (1.1)
Moderate	4 (8.3)	3 (6.4)	7 (7.4)
Severe	2 (4.2)	1 (2.1)	3 (3.2)
External Head/Neck			
Mild	4 (8.3)	0	4 (4.2)
Moderate	8 (16.7)	9 (19.1)	17 (17.9)
Severe	2 (4.2)	0	2 (2.1)
Cutaneous			
Mild	2 (4.2)	4 (8.5)	6 (6.3)
Moderate	23 (47.9)	17 (36.2)	40 (42.1)
Severe	9 (18.8)	0	9 (9.5)

Source: Summary Table 14.2.1.1

Abbreviations: ITT = intent-to-treat

[a] Symptom complex identification taken from patient predose diary.

[b] Denominator used to calculate percents is based on the number of patients in the treatment group or overall with available diary data.

[c] A patient could have more than one symptom complex at baseline.

[d] Patient 413001 mistakenly used a test Logpad and had no patient diary data available.

Number of baseline attacks

The table below shows Symptom Complexes and Emerging Symptoms in both EDEMA 3 and EDEMA4.

In contrast to EDEMA 3, more than 50% of patients in each arm of EDEMA 4 had only one presenting symptom complex.

	EDEMA3-DB		EDEMA4	
# symptom complexes at Baseline	DX-88 N=36 n (%)	Placebo N=36 n (%)	DX-88 N=48 n (%)	Placebo N=48* n (%)
1	19 (52.8)	26 (72.2)	28 (58.3)	27 (57.4)
2	16 (44.4)	5 (13.9)	12 (25.0)	13 (27.7)
3	0 (--)	4 (11.1)	5 (10.4)	6 (12.8)
4	1 (2.8)	1 (2.8)	2 (4.2)	1 (2.1)
5	0 (--)	0 (--)	1 (2.1)	0 (--)
# patients with emerging symptom complexes at up to 4 hours post treatment	1 (2.8)	3 (8.3)	2 (4.2)	7 (14.9)

Source: Supplemental Efficacy Analysis Listings 7.1 and 7.2; EDEMA3-DB Listing 16.2.6.1.4; EDEMA4 Listing 16.2.8.7.5.1
*Placebo patient 413001 did not complete a patient diary and therefore no symptom complex data are available. Percentages are based on N=47.

Numbers analysed

Ninety-six (96) patients were planned, per protocol Amendment 2; 96 patients were analyzed for safety; 96 patients were analyzed in the intent-to-treat (ITT) population; 95 patients were analyzed in the Per Protocol (PP) population.

Outcomes and estimation

Primary Endpoint

Change From Baseline in the MSCS Score at 4 Hours Post-Dosing

Table 27. MSCS Score and Change From Baseline at 4 Hours Post-dosing (ITT Population)

		Baseline			4 Hours Post-dosing			Change From Baseline		
Time Point Treatment	N	Median (IQR)	(Min, Max)	Mean (SD)	Median (IQR)	(Min, Max)	Mean (SD)	Median (IQR)	(Min, Max)	Mean (SD)
Baseline										
Ecallantide	48	2.0 (2.0, 2.5)	(1.5, 3.0)	2.2 (0.50)						
Placebo	47	2.0 (2.0, 2.0)	(1.5, 3.0)	2.0 (0.40)						
4 Hours										
Ecallantide	47	2.0 (2.0, 2.5)	(1.5, 3.0)	2.2 (0.50)	1.0 (1.0, 2.0)	(0.0, 3.0)	1.4 (0.75)	-1.0 (-1.0, 0.0)	(-2.0, 0.0)	-0.8 (0.63)
Placebo	42	2.0 (2.0, 2.0)	(1.5, 3.0)	2.0 (0.35)	2.0 (1.0, 2.0)	(0.0, 3.0)	1.6 (0.77)	0.0 (-1.0, 0.0)	(-2.0, 1.5)	-0.4 (0.82)
P value[a]									0.010	

Source: Summary Table 14.2.3.1

Abbreviations: IQR = interquartile range (25th, 75th percentiles); ITT = intent-to-treat; max = maximum; min = minimum; MSCS = Mean Symptom Complex Severity; SD = standard deviation

[a] Blocked Wilcoxon rank sum test.

The mean change in MSCS score from baseline to 4 hours is -0.8 versus -0.4 in the active and placebo groups. The relevance of a change of 0.4 in MSCS is small and the meaning difficult to interpret.

However, only 89 patients (47 patients in the ecallantide and 42 patients in the placebo group) were evaluable for the primary efficacy endpoint. The primary efficacy analysis was performed without any imputation on these 89 patients, not on all randomized patients. The Applicant has presented a wide range of suitable sensitivity analyses, all of which reach statistical significance. Given the robustness of the data to the missing data, this issue has been adequately addressed.

Secondary endpoints

TOS at 4 Hours Post-dosing, Based on the Patient's Severity Assessment of Symptom Complexes at Baseline

Table 28. TOS at 4 Hours Post-dosing (ITT Population)

	Treatment Group	
Statistic	Ecallantide (N=48)	Placebo (N=48)
n	47	42
Median	50.0	0.0
IQR	(0.0, 100.0)	(-50.0, 50.0)
(Min, max)	(-66.7, 100.0)	(-100.0, 100.0)
Mean (SD)	53.4 (49.70)	8.1 (63.18)
P value[a]	0.003	

Source: Summary Table 14.2.4.1

Abbreviations: IQR = interquartile range (25th, 75th percentiles); ITT = intent-to-treat; max = maximum; min = minimum; SD = standard deviation; TOS = Treatment Outcome Score

[a] Blocked Wilcoxon rank sum test.

The problems with interpretation of the TOS and the high numbers in both groups who required medical interventions and additional ecallantide doses make this secondary endpoint unclear in terms of clinical relevance.

Time to Significant Improvement in Overall Response

The median time to significant improvement was not reached for either treatment group, and the differences in the distributions for the time to significant improvement was not statistically significant between treatment groups (Wilcoxon P=0.117, log-rank P=0.102).

Table 29. Time to Significant Improvement in Overall Response (Kaplan-Meier) (ITT Population)

	Treatment Group	
	Ecallantide (N=48)	Placebo (N=48)[e]
Patients with significant improvement[a], n (%)	22 (45.8)	12 (25.5)
Time to significant improvement, minutes[a]		
Estimated mean[b]	184.3	154.3
Estimated median[c]	--	--
IQR[c]	165.3, --	165.6, --
P values[d]	0.117, 0.102	

Source: Summary Table 14.2.5.1

Abbreviations: IQR = interquartile range (25th, 75th percentiles); ITT = intent-to-treat

[a] Significant improvement was defined as an overall assessment of "a lot better or resolved."

[b] The mean time to onset of significant improvement may be biased due to censoring at 4 hours.

[c] -- indicates was not reached

[d] Wilcoxon and log-rank tests, respectively

[e] Diary information for Patient 413001 is not available. The patient is considered not evaluable and excluded from the analysis. Percentages for placebo treatment group are based on a denominator of 47.

The outcome of time to significant improvement is the most important endpoint for this trial and this was not met. Although a secondary endpoint, time to significant improvement is considered the most clinically important in terms of treating a laryngeal attack. The efficacy is therefore not considered robust.

Proportion of Patients with a Successful Response at 4 Hours Post-dosing, Based on the Change from Baseline in the MSCS Score

A successful response was defined as improvement in existing laryngeal symptom complex, stabilization of an existing peripheral symptom complex, or a change from baseline in the MSCS score at 4 hours of at least -1.0. As presented in Table 30, for the ITT population, 93.8% of the ecallantide group (45 of 48 patients) and 59.6% of the placebo group (28 of 47 patients with available diary data) had a successful response assessment at 4 hours after dosing.

Table 30. Proportion of Patients with a Successful Response at 4 Hours Post-dosing (ITT Population)

	n	%	Parameter Estimate	*P* Value[a]	Odds Ratio
Patients with a successful response[b]			-	-	-
Ecallantide (N=48)	45	93.8			
Placebo (N=48)[c]	28	59.6			
Variable	-	-			
Treatment (ecallantide vs placebo)	-	-	1.207	0.001	11.18
Attack location (laryngeal vs all others)	-	-	1.069	0.022	8.49
Prior use of ecallantide (yes vs no)	-	-	0.060	0.835	1.13

Source: Summary Table 14.2.7.1

Abbreviations: ITT = intent-to-treat

[a] *P*-value from the logistic regression model

[b] A successful response defined as improvement in existing laryngeal symptom complex, stabilization of existing peripheral symptom complex, or a change in baseline at 4 hours MSCS of at least -1.0.

[c] Diary information for Patient 413001 is not available. The patient is considered not evaluable and excluded from the analysis. Percentages for placebo treatment group are based on a denominator of 47.

Although the applicant states that patients who received ecallantide and patients who had attacks at locations other than laryngeal were more likely to have a successful response at 4 hours than patients who received placebo or patients who had laryngeal attacks; the definition of a responder is considered weak and non-conservative, the results are based on "overall response" and in conclusion the clinical relevance of these results is not clear.

Maintenance of Significant Improvement in Overall Response

Table 31. Proportion of Patients Maintaining a Significant Improvement in Overall Response Through 24 Hours (ITT Population)

					Odds Ratio Estimate		
						95% CI	
	Ecallantide (N=48)	Placebo (N=48)[a]	Coefficient Estimate	*P* Value[b]	Point Estimate	Lower	Upper
Proportion of Patients Maintaining Significant Improvement, n (%)	21 (43.8)	10 (21.3)					
Parameter							
Treatment (ecallantide vs placebo)			0.533	0.022	2.90	1.17	7.20
Attack location (laryngeal vs all others)			0.137	0.754	1.32	0.24	7.29
Prior use of ecallantide (yes vs no)			0.188	0.418	1.46	0.59	3.62

Source: Summary Table 14.2.6.1

Abbreviations: ITT = intent-to-treat

Note: Maintenance of significant improvement is defined as continued patient response to overall assessment as "a lot better or resolved" prior to or on the 4-hour post-dosing assessment continuously through the 24-hour post-dosing assessment.

[a] Diary information for Patient 413001 is not available. The patient is considered not evaluable and excluded from the analysis. Percentages for placebo treatment group are based on a denominator of 47.

[b] P value from logistic regression model

Because less than half of cases in the active arm had a significant improvement (in overall response) through 24 hours, this data is not considered to provide convincing and consistent evidence of efficacy.

Tertiary Efficacy Endpoints

Because of the problems in assessing the efficacy with MSCS and PRO and *Successful Response* at earlier time points, the 24 hour data is not considered supportive. The results are presented below.

Durability of Response at 24 Hours Post-dosing - Change From Baseline in the MSCS Score

TOS at 4 Hours Post-dosing, Based on the Investigator's Severity Assessment of Symptom Complexes at Baseline

The results for the TOS at 4 hours based on the Investigator's severity assessment are similar to those based on the patient's severity assessment.

Proportion of Responders at 4 Hours Post-dosing, Based on the TOS

A significantly larger proportion of patients having a successful response was observed for the ecallantide group (P=0.011). In the ITT population, 45.8% of the ecallantide group (22 of 48 patients) and 19.1% of the placebo group (9 of 47 patients with available diary data) had a TOS ≥70 at 4 hours after dosing.

A significantly larger proportion of patients having a successful response was observed for the ecallantide group (P<0.001). In the ITT population, 68.8% of the ecallantide group (33 of 48 patients) and 27.7% of the placebo group (13 of 47 patients with available diary data) had a TOS ≥50 at 4 hours after dosing.

Table 32. Proportion of Responders at 4 hours based on TOS ≥70 and TOS ≥50 (ITT Population)

	n	%	Parameter Estimate	*P* Value[a]	Odds Ratio
Patients with TOS ≥70			-	-	-
Ecallantide	22	45.8			
Placebo[b]	9	19.1			
Variable	-	-			
Treatment	-	-	0.613	0.011	3.41
Attack location (laryngeal vs all others)	-	-	-0.407	0.445	0.44
Prior use of ecallantide (yes vs no)	-	-	0.227	0.345	1.58
Patients with TOS ≥50			-	-	-
Ecallantide	33	68.8			
Placebo[b]	13	27.7			
Variable	-	-			
Treatment	-	-	0.827	<0.001	5.23
Attack location (laryngeal vs all others)	-	-	0.022	0.969	1.05
Prior use of ecallantide (yes vs no)	-	-	-0.057	0.811	0.89

Source: Summary Tables 14.2.11 and 14.2.12

Abbreviations: ITT = intent-to-treat; TOS=Treatment Outcome Score

[a] *P* value from the logistic regression model

[b] Diary information for Patient 413001 is not available. The patient is considered not evaluable and excluded from the analysis. Percentages for placebo treatment group are based on a denominator of 47.

Time to Onset of Sustained Improvement in Overall Response

Sustained improvement in overall response is an assessment by the patient of "a little better" or "a lot better or resolved" in overall well-being for a continuous duration of ≥45 minutes during the 4-hour period after dosing. Table 33 summarizes the time to onset of sustained improvement in overall response for the ITT population by treatment group. In the ITT population, 66.7% of the ecallantide group (32 of 48 patients) and 38.3% of the placebo group (18 of 47 patients with available diary data) showed a sustained improvement. The median time to onset of sustained improvement was 135.6 minutes for the ecallantide group and was not reached for the placebo group. The differences in the

distributions of time to onset of sustained improvement was not statistically significant between treatment groups using the Wilcoxon test (P=0.150) but did reach marginal significance using the log-rank test (P=0.050).

Table 33. Time to Onset of Sustained Improvement in Overall Response (Kaplan-Meier) (ITT Population)

	Treatment Group	
	Ecallantide (N=48)	Placebo (N=48)[e]
Patients with sustained improvement, n (%)[a]	32 (66.7)	18 (38.3)
Time to onset of sustained improvement, minutes[a]		
Mean[b]	141.3	142.9
Median[c]	135.6	--
IQR[c]	(60.3, --)	(55.4, --)
P values[d]	0.150, 0.050	

Source: Summary Table 14.2.13.1

Abbreviations: IQR = interquartile range (25th, 75th percentiles); ITT = intent-to-treat

[a] Sustained improvement was defined as the first time (in minutes) throughout the first 4 hours post-dosing that a patient had an improvement in overall response ("a little better" or "a lot better or resolved") for a continuous duration of ≥45 minutes.

[b] The mean time to onset of sustained improvement may be biased due to censoring at 4 hours.

[c] -- indicates not reached

[d] Wilcoxon and log-rank tests, respectively.

[e] Diary information for Patient 413001 is not available. The patient is considered not evaluable and excluded from the analysis. Percentages for placebo treatment group are based on a denominator of 47.

The results for this tertiary endpoint are not considered robust, when taking into account the definition of the overall response and the presentation of the data in terms of overall response.

Patients Receiving Medical Intervention during an attack

The majority of medical interventions that were administered in both treatment groups were emergency medications (31.3% in the ecallantide group [15 of 48] and 50.0% in the placebo group [24 of 48]), including new or increased doses of 5-HT3 receptor antagonists, opioids, anti-emetic medications, or C1-INH, or the open-label administration of ecallantide (for SUAC or as Dose B). Patients could receive more than 1 medical intervention.

Within 4 hours of treatment the percentage of subjects requiring intervention was 2% and 17% in the ecallantide and placebo arms respectively. Within 24 hours however there was a high rate of intervention in both arms at 35% (31% emergency) and 50% (all emergency) for ecallantide and placebo arms respectively.

This is of concern and suggests that there is either insufficient efficacy, a rapid loss of efficacy, or rebound.

Assessment of Response to Open-Label Dosing

Table 34. MSCS Change from Baseline at 4 hours After Dose B Compared to MSCS Change from Baseline at 4 Hours After the Initial Dose

	Ecallantide (N=14) n (%)	Placebo (N=20) n (%)
Better	9 (64.3)	10 (50.0)
Same	5 (35.7)	5 (25.0)
Worse	0	1 (5.0)
Not Evaluable	0	4[a] (20.0)

Source: Table 32

[a] Includes Patient 413001 who has neither the 4-hour evaluation after the initial dose nor after Dose B.

The data from Dose B is difficult to interpret in view of the MSCS score and as 4/20 of the results from the original placebo group were not evaluable for MSCS. The applicant provided more detailed data on these 4 cases, which showed that 1 patient has a response, 1 had some improvement, 1 had no response and the 4 hour data is missing for the 4th patient.

OPEN-LABEL TREATMENT FOR SEVERE UPPER AIRWAY COMPROMISE

Patients who experienced SUAC within 0 to 4 hours after initial dosing may have been administered an open-label dose of ecallantide. The response to SUAC dosing, based on the change from baseline in the MSCS score at 4 hours after SUAC administration was to be assessed. Table 35 presents a description of each case of SUAC dosing. The SUAC dose for all patients was administered approximately an hour and a half after the administration of the initial double-blind dose.

A decrease from baseline in the MSCS score represents an improvement in symptom severity.

Of the 48 patients who received ecallantide as their initial treatment, 1 (2.1%) was treated for SUAC. This patient experienced a change from baseline of -1.0 in MSCS score 4 hours after SUAC administration.

Of the 48 patients who received placebo as their initial treatment, 3 (6.3%) were treated for SUAC. For patients who initially received placebo, SUAC represents the first time they receive ecallantide during the study. At 4 hours after SUAC dosing, 1 patient had a change from baseline in MSCS score of -1.0; the other 2 patients had increases from baseline in MSCS score (0.5 and 1.0).

Table 35. Patients Receiving Open-Label Ecallantide for SUAC

Treatment	Patient	Symptom Complex Involved in HAE Attack (Severity)	Timing of SUAC Dose	Change from Baseline MSCS Score at 4 Hours After Open Label SUAC Dose
Ecallantide	411001	Internal Head/Neck (moderate) External Head/Neck (mild)	1 hr 41 min	-1.0
Placebo	417014	Internal Head/Neck (mild) External Head/Neck (moderate)	1 hr 48 min	0.5
	439003	Internal Head/Neck (moderate)	1 hr 32 min	-1.0
	443001	Internal Head/Neck (moderate)	1 hr 46 min	1.0

Source: Summary Table 14.3.5.2, Appendices 16.2.6.3 and 16.2.8.7.5.1

Abbreviations: HAE = hereditary angioedema; SUAC = severe upper airway compromise

50% of those receiving open label ecallantide for SUAC in EDEMA4 deteriorated based on the MSCS score. This does not support efficacy in the most important location for HAE attacks.

Although many outcomes were studied it is time to onset of significant response that is the most important one assessed in this trial. In addition other outcomes namely: the percentage with successful outcomes at 4 hrs and the percentage who required emergency and/or additional medical interventions are important. These outcomes should show a clear difference between the active and placebo arm that is convincing and consistent and this is not considered to have been demonstrated.

In addition the assessment of efficacy in this study is complicated by the MSCS score, the TOS severity score, the definition of a successful response and the use of "overall score" rather than location-specific score. At the day 120 responses it was clarified that 3 subjects (1 in EDEMA 3 and 2 in EDEMA 4 in the ecallantide arm) developed emerging symptom complexes. Another area of uncertainty is whether those with multiple symptom complexes had differences in their qualitative responses at each location. Efficacy has not been considered to have been demonstrated.

Clinical studies in special populations

No studies specifically designed for special populations were performed.

However an overview of results from the paediatric cases is provided below.

During the clinical development program in HAE, paediatric patients ≥10 years of age were enrolled in 5 studies: EDEMA1, EDEMA2, EDEMA3, EDEMA4, and DX-88/19 (ongoing continuation study). A total of 34 unique paediatric patients have been enrolled in theses 5 studies and received their first dose of study drug before the age of 18 years. Six of the paediatric patients were younger than 12 years of age at the time of their first exposure to ecallantide. One patient was 9 years of age at the time of the first dose; this patient was granted an exception to the entrance criteria (10 years of age or above). A total of 20 paediatric patients have been included in the double-blind, placebo-controlled studies.

Table 36: Description of Efficacy Results for Patients Aged 10 Through 17 in Without a Placebo Comparison (Completed Studies) (n=18)

Patient	Age[1]	Description of Efficacy	Positive Response[2]
8805003088	10	EDEMA2: Beginning of improvement in 26 minutes	Yes
8804017015	11	EDEMA1: Significant improvement in 5 minutes EDEMA2: Beginning of improvement in 15 minutes, significant improvement in 60 minutes	Yes
8805024099	11	EDEMA2: Beginning of improvement in ≤43 minutes in 3 of 5 treated attacks. No improvement in 2 of 5 attacks. Significant improvement in ≤216 minutes in 2 of 5 attacks, no significant improvement in 3 attacks	No
8804023001	12	EDMEA1: Beginning of improvement in 200 minutes EDEMA2: Beginning of improvement in ≤120 minutes in 16 of 18 attacks, not achieved in 2 attacks. Significant improvement in ≤240 minutes in 14 of 18 attacks, no significant improvement 4 attacks.	Yes
8814372004	12	EDEMA3: Change in MSCS of -1.0 and TOS of 50	Yes
8805003099	13	EDEMA2: Beginning of improvement not achieved	No
8804017001	13	EDEMA1: Beginning of improvement in 60 minutes, significant improvement in 90 minutes EDEMA2: Beginning of improvement in ≤45 minutes in 6 of 7 attacks, not achieved in 1 attack. Significant improvement in 3 of 7 attacks in ≤215 minutes, no significant improvement in 4 attacks.	Yes
8804013006	14	EDEMA1: Beginning of improvement in 20 minutes, significant improvement in 75 minutes	Yes
8805019001	14	EDEMA2: Beginning of improvement in 15 minutes, significant improvement in 90 minutes	Yes
8805015006	15	EDEMA2: Beginning of improvement in 40 minutes, significant improvement not achieved	Yes
8805022008	15	EDEMA2: Beginning of improvement not achieved, significant improvement not achieved EDEMA3: Average change in MSCS score of -0.25 and average TOS of 0 in 2 attacks	No
8814372001	15	EDEMA3: Change in MSCS of -1.0 and TOS of 50	Yes
8805003004	16	EDEMA2: Beginning of improvement in ≤120 minutes in 2 of 3 attacks, significant improvement in ≤240 minutes in 1 of 3 attacks	Yes
8804018002	16	EDEMA1: Beginning of improvement in 15 minutes, significant improvement in 15 minutes	Yes
8805027001	16	EDEMA2: Beginning of improvement not achieved, significant improvement not achieved	No
8820424001	16	EDEMA4: Change in MSCS of -1.0 and TOS of 100	Yes
8804018004	17	EDEMA1: Beginning of improvement in 5 minutes, significant improvement in 15 minutes EDEMA2: Beginning of improvement in ≤4 minutes in 2 of 3 attacks, significant improvement in ≤62 minutes in 2 of 3 attacks	Yes
8820404011	17	EDEMA4: Change in MSCS of -1.3 and TOS of 100	Yes

Note: a duration of 0 minutes indicates that improvement occurred during the infusion.
[1] Age at the time patient first received treatment with study drug
[2] Adjudicated as per Table 2.7.3.33
Source: LPPs

The efficacy data for children aged 12 to 18 years (n=25) as provided in the table above is without placebo controlled groups and has the same problems of interpretation as do the main pivotal trials. In addition various different dosages have been used with different modes of administration.

Moreover, the pharmacokinetic data is in children is insufficient to justify using the same dose in children as in adults.

Analysis performed across trials (pooled analyses AND meta-analysis)

Pooled meta-analysis was performed by the applicant across the trials as presented below. However in view of the problems with the individual phase 3 trials this analysis is not considered supportive.

Table 37 . Change from Baseline in MSCS Score at 4 and 24 Hours:Controlled Phase 3 Studies—Results from CSRs

Statistics	Ecallantide	Placebo	Ecallantide	Placebo	Ecallantide	Placebo	*P Value*[a]
EDEMA4 ITT Population							
	Baseline		**4 Hours**		**Change**		
N	47	42	47	42	47	42	0.010
Median	2.0	2.0	1.0	2.0	-1.0	0.0	
IQR	2.0, 2.5	2.0, 2.0	1.0, 2.0	1.0, 2.0	-1.0, 0.0	-1.0, 0.0	
Range	1.5, 3.0	1.5, 3.0	0, 3.0	0, 3.0	-2.0, 0.0	-2.0, 1.5	
Mean	2.18	1.99	1.38	1.62	-0.81	-0.37	
SD	0.50	0.35	0.75	0.77	0.63	0.82	
	Baseline		**24 Hours**		**Change**		
N	32	24	32	24	32	24	0.039
Median	2.0	2.0	0.3	1.0	-1.6	-1.0	
IQR	2.0, 2.0	2.0, 2.0	0.0, 1.0	0.0, 1.8	-2.0, -1.0	-2.0, -0.6	
Range	1.5, 3.0	1.5, 3.0	0.0, 2.0	0.0, 3.0	-2.7, 0.0	-2.0, 1.0	
Mean	2.1	2.1	0.6	1.0	-1.5	-1.1	
SD	0.45	0.41	0.66	0.89	0.63	0.84	
EDEMA3-DB ITT-as-Randomized Population							
	Baseline		**4 Hours**		**Change**		
N	36	36	36	36	36	36	0.014
Median	2.00	2.00	1.00	2.00	-1.00	-0.50	
IQR	2.00, 2.38	2.00, 3.00	0.50, 2.00	1.00, 2.50	-1.50, 0.00	-1.00, 0.00	
Range	1.50, 3.00	1.25, 3.00	0.00, 3.00	0.00, 3.00	-3.00, 1.50	-1.67, 1.75	
Mean	2.15	2.26	1.26	1.75	-0.88	-0.51	
SD	0.49	0.56	0.96	0.90	1.11	0.68	
	Baseline		**24 Hours**		**Change**		
N	33	33	33	33	33	33	0.041
Median	2.0	2.0	1.0	1.3	-1.0	-0.5	
IQR	2.0, 2.0	2.0, 3.0	0.5, 2.0	1.0, 3.0	-2.0, 0.0	-1.0, 0.0	
Range	1.0, 3.0	1.0, 3.0	0.0, 3.0	0.0, 3.0	-3.0, 1.5	-2.0, 2.0	
Mean	2.07	2.21	1.20	1.74	-0.87	-0.46	
SD	0.50	0.59	1.02	1.01	1.14	1.07	

Source: EDEMA4 CSR Summary Table 14.2.3.1 and 14.2.8.1, EDEMA3-DB CSR Summary Table 14.2.2.1.1; Supplemental Efficacy Analysis Table 3.8.5.2 and 3.8.5.3.1.

IQR = interquartile range; SD = standard deviation

[a] Blocked Wilcoxon rank sum test incorporating stratification variables (prior exposure to ecallantide and primary attack location).

Integrated analysis of time to response parameters was provided in the day 120 responses.

The applicant proposed that the most relevant time to response parameter for different location attacks were as follows:

- Laryngeal: Time to significant improvement is a key threshold due to the life-threatening nature of this attack.
- Abdominal: Time to sustained improvement is more clinically relevant for patients due to the transient nature for symptom improvement at this attack location and the avoidance of surgery due to a misdiagnosis.
- Peripheral (genital/buttocks, external head/neck, and cutaneous symptom complexes):

  Time to the beginning of improvement is more clinically relevant threshold as these attacks are known to take longer to resolve.

Laryngeal attacks

When time to significant improvement for laryngeal attacks is assessed only taking into account the laryngeal attack (Fig 150-1) and not the overall response (Fig 83-6) , the difference between the ecallantide and placebo arms is limited and the number of censored cases is similar in both arms. As laryngeal attacks are the most severe and require urgent treatment the data provided does not support the use of ecallantide in laryngeal oedema.

In addition the difference between the two analyses (location-specific or overall) raise further uncertainties about the efficacy presented for abdominal and peripheral attacks below.

Figure 150-1. Time to Significant Improvement for Patients with a Moderate or Severe Laryngeal Attack

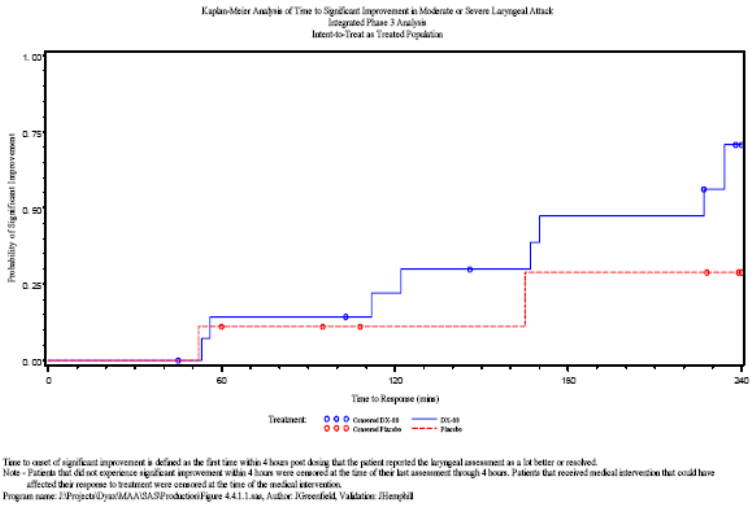
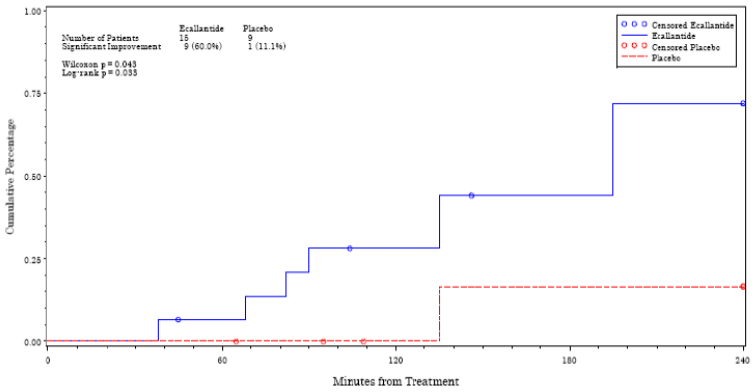


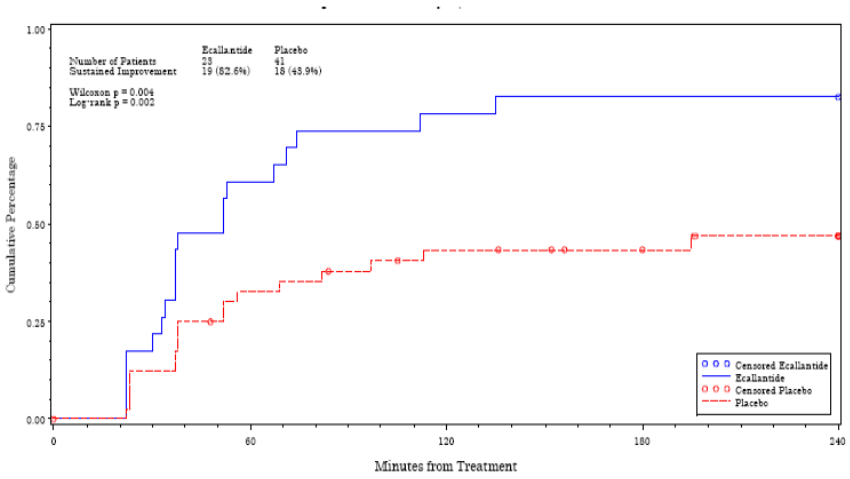
Figure 83-6. Time to Significant Improvement for Patients Whose Primary Attack Location Was Laryngeal (IP3, ITT-as-Treated)



Source: Supplemental Efficacy Analysis Figure 3.4.1.5

Abdominal Attacks

Figure 83-9. Time to Sustained Improvement for Patients Whose Primary Attack Location Was Abdominal (IP3, ITT-as-Treated)

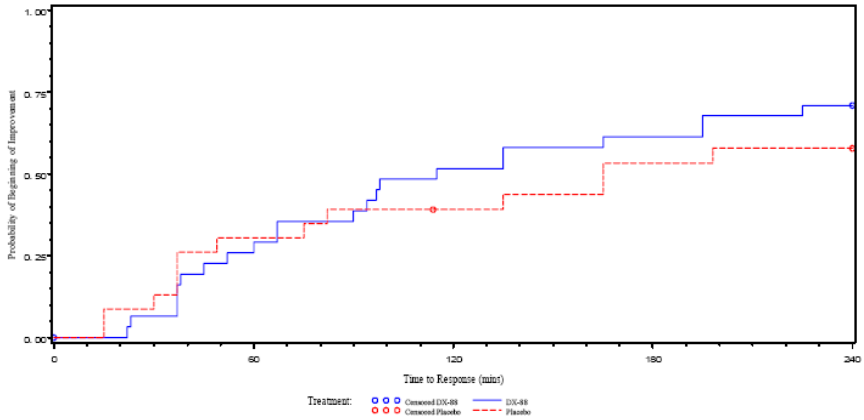


Source: Supplemental Efficacy Analysis Figure 3.4.2.4

The evidence for efficacy is abdominal attacks is much stronger although note that this data is presented as overall response. However since one of the most prominent symptoms in abdominal attacks is abdominal pain, the efficacy evaluation may be confounded by factors or medications that reduce pain but which possibly do not have a comparable effect on swelling, and thus confounding a comparison across attack locations.

Peripheral attacks

Figure 83-12. Time to the Beginning of Improvement for Patients Whose Primary Attack Location Was Peripheral (IP3, ITT-as-Treated)



Time to onset of beginning of improvement is defined as the first time (in minutes) post dosing that the patient reported the overall assessment as 'better' or 'a lot better or resolved'.
Note - Patients that did not experience beginning of improvement within 4 hours were censored at the time of their last assessment through 4 hours. Patients that received a medical intervention that could have affected their response to treatment were censored at the time of the medical intervention.
Program name: J:\Projects\Dyax\MAA\SAS\Production\Figure 4.5.2.sas, Author: JHemphill, Validation:

Source: Supplemental Efficacy Analysis Figure 4.5.3

No difference between active and placebo is seen in the time to response parameter considered the most relevant by the applicant.

In summary efficacy in time to response parameters is demonstrated for abdominal attacks, not considered convincing for laryngeal attacks and not present for peripheral attacks. This is unexpected and raises concerns about the adequacy of the posology, the MoA of ecallantide and the duration of effect.

Supportive study(ies)

One controlled Phase 2 study (EDEMA1) and 2 uncontrolled Phase 2 studies (EDEMA2 and EDEMA0) were conducted in patients with HAE. In addition, patients received multiple open-label doses of ecallantide in the EDEMA3-RD study.

EDEMA3-RD was a repeat-dosing part of the EDEMA3 study was an open-label study designed to evaluate the effects of repeated treatments of ecallantide in patients who experienced multiple acute attacks of HAE.

The total ITT patient population was N=66

Primary Efficacy Endpoint Analysis was TOS at 4 Hours Post-dosing over Multiple Treatment Episodes

(ITT Population)

Table 38. TOS at 4 Hours Post-dosing over Multiple Treatment Episodes (ITT Population)

Treatment Episode	N	Median (IQR)	Mean (SD)
1	18	68.8 (50, 100)	71.3 (28.85)
2	51	100.0 (50, 100)	73.3 (44.9)
3	30	100.0 (70, 100)	81.9 (28.52)
4	21	100.0 (38, 100)	81.2 (24.53)
5	11	100.0 (0, 100)	48.5 (68.5)
6	9	60.0 (50, 100)	60.4 (49.26)

Source: Summary Table 14.2.1.1

IQR=interquartile range, SD=standard deviation

Secondary Efficacy Endpoint Analyses

Time to Significant Improvement in Overall Response

Table 39. Time to Significant Improvement in Overall Response over Multiple Treatment Episodes (ITT Population)

		Significant Improvement[a]	
Treatment Episode	N	Achieved n (%)	Median Time to (min)
1	18	10 (55.6)	229.5
2	51	37 (72.5)	127.5
3	30	24 (80.0)	94.0
4	21	14 (66.7)	112.0
5	11	6 (54.5)	195.0
6	9	5 (55.6)	135.0

Source: Summary Table 14.2.3.3

[a] Significant improvement was defined as an overall response assessment of "a lot better or resolved."

Discussion on clinical efficacy

The important efficacy endpoint for a treatment of HAE is a quick response to treatment and this should be evident in the majority of patients treated and be sustained. This is particularly important in a life-threatening attack. The PROs used in the pivotal trials are complex and considered flawed such that events such as emerging symptom complexes or transient worsening of an attack (presenting or otherwise) only contribute to the PROs in a quantitative way. These events are failure of therapy and may even constitute a safety signal. In view of the similarity of the clinical picture of HAE systemic allergic reaction (seen in 18 subjects), the possibility to confuse an allergic reaction with worsening of the presenting attack or the development of a new HAE attack exists. The high proportion of patients who were censored in the active group does not support efficacy. The difference in time to response parameters in different locations is of serious concern, raising further concerns about the MoA of ecallantide. The increased number of AEs reported as "HAE" in the ecallantide group compared with placebo from day 0-2 also suggests either lack of efficacy or rebound.

Conclusions on clinical efficacy

Efficacy has not been clearly demonstrated for the clinically relevant secondary endpoint of time to response in the pivotal trials. The PROs are considered problematic and do not reflect treatment failure appropriately. The difference in efficacy in difference locations is unexpected and of serious concern.

Clinical safety

Patient exposure

Table 40. Total Human Exposure to Ecallantide in the HAE Development Program

Population	Number of Subjects Who Received Ecallantide	Number of Ecallantide Doses[a]
Total number of subjects overall (HAE and healthy subjects)[b]	350	1387
Total number of HAE patients overall[c]	286	1246
Total number in the Safety Evaluation		
All HAE Studies[d]	283	1217
Completed HAE Studies[e]	219	609
Double-Blind HAE SC[f]	100	125
Healthy Subjects	62	139
Rechallenge	6	6
Compassionate use	8	17

Source: DX-88/1 CSR; DX-88/2 CSR; DX-88/4 CSR; DX-88/5 CSR; DX-88/6 CSR; DX-88/13 CSR; DX-88/14-DB CSR; DX-88-14 RD CSR; DX-88/15 CSR; DX-88/20 Listing 16.2.5; DX-88/19 Listing 16.2.1.1, and 16.2.4.1; CS88/19 Study Drug Administration Listing; and Module 5.3.5.3 LPP.

Table 41. Total Ecallantide Exposure: All HAE Studies Population

	Ecallantide (N=283)			
	N	(%)	Min; Max Total Cumulative Dose (mg)	Min; Max Duration
Number of patients with[a]:				
1 dose	95	(33.6)	8.5; 89.6	1 day
2 to 4 doses	110	(38.9)	27.9; 153.2	1 day; 35 months, 28 days
5 to 9 doses	42	(14.8)	80.2; 310.8	20 days; 67 months, 16 days
>9 doses	36	(12.7)	169.2; 1043.5	3 months, 3 days; 74 months, 9 days

Source: Supplemental Safety Analysis Table 3.3, DX-88/19 Study Drug Administration Listing, Longitudinal Patient Profiles

Abbreviations: HAE=hereditary angioedema.

[a] Exposure is defined as the cumulative number of doses across all studies in this analysis population; categories are mutually exclusive. Only ecallantide exposure (including ecallantide doses for SUAC and as Dose B) for patients who received both ecallantide and placebo is included.

286 patients is considered a limited safety database when the majority have had less than 5 doses.

Adverse events

Table 42. Overall Summary of Adverse Events in HAE Clinical Studies

	All HAE Studies		Double-Blind HAE SC Population			
	Ecallantide (N=283)		Ecallantide (N=100)		Placebo (N=81)	
	n	(%)	n	(%)	n	(%)
At least 1 TEAE	198	(70.0)	36	(36.0)	28	(34.6)
Drug-related TEAEs	98	(34.6)	15	(15.0)	11	(13.6)
At least 1 SAE	44	(15.5)	3	(3.0)	3	(3.7)
Drug-related SAEs	11	(3.9)	0	(0.0)	0	(0.0)
Deaths	2	(0.7)	0	(0.0)	0	(0.0)
Withdrew from study due to a TEAE	2	(0.7)	0	(0.0)	0	(0.0)
Withdrew from study due to a SAE	2	(0.7)	0	(0.0)	0	(0.0)
Withdrew from study due to a drug-related SAE	1	(0.3)	0	(0.0)	0	(0.0)

Source: ISS Summary Tables 5.1.2, 5.3.2, ISS Listing 2.1; Supplemental Safety Analysis Tables 1.4, 5.1.4, 5.3.4, 6.1.4, 6.3.4, DX-88/19 Table 14.3.2.1

Table 43 Treatment-Emergent Adverse Events Occurring in ≥2% of Patients in Either Treatment Group: Double-Blind HAE SC Population

System Organ Class Preferred Term	Ecallantide (N=100)		Placebo (N=81)	
	n	(%)	n	(%)
Patients with ≥1 TEAE	36	(36.0)	28	(34.6)
Congenital, Familial and Genetic Disorders	3	(3.0)	4	(4.9)
Hereditary Angioedema	3	(3.0)	4	(4.9)
Gastrointestinal Disorders	14	(14.0)	8	(9.9)
Abdominal Pain	1	(1.0)	2	(2.5)
Abdominal Pain Upper	2	(2.0)	0	(0.0)
Diarrhea	4	(4.0)	3	(3.7)
Nausea	5	(5.0)	1	(1.2)
Vomiting	0	(0.0)	3	(3.7)
General Disorders and Administration Site Conditions	10	(10.0)	4	(4.9)
Fatigue	2	(2.0)	0	(0.0)
Injection Site Pain	2	(2.0)	0	(0.0)
Pyrexia	4	(4.0)	0	(0.0)
Infections and Infestations	9	(9.0)	6	(7.4)
Nasopharyngitis	3	(3.0)	0	(0.0)
Upper Respiratory Tract Infection	0	(0.0)	2	(2.5)
Investigations	0	(0.0)	2	(2.5)
Prothrombin Time Prolonged	0	(0.0)	2	(2.5)
Nervous System Disorders	12	(12.0)	8	(9.9)
Dizziness	2	(2.0)	1	(1.2)
Headache	8	(8.0)	6	(7.4)
Respiratory, Thoracic and Mediastinal Disorders	5	(5.0)	1	(1.2)
Pharyngolaryngeal Pain	2	(2.0)	0	(0.0)
Skin and Subcutaneous Tissue Disorders	5	(5.0)	3	(3.7)
Erythematous Rash	2	(2.0)	0	(0.0)

Source: ISS Summary Table 5.1.2.

Serious adverse events and deaths

Table 44. Treatment-Emergent Serious Adverse Events: All HAE Studies Population

System Organ Class Preferred Term	Ecallantide (N=283) N (%)
Patients with ≥1 SAE	44 (15.5)
Congenital, Familial and Genetic Disorders	23 (8.1)
Hereditary Angioedema	23 (8.1)
Gastrointestinal Disorders	7 (2.5)
Abdominal Pain	2 (0.7)
Colitis	1 (0.4)
Diarrhea	1 (0.4)
Gingival Recession	1 (0.4)
Hematochezia	1 (0.4)
Pancreatitis	1 (0.4)
Vomiting	1 (0.4)
General Disorders and Administration Site Conditions	4 (1.4)
Adverse Drug Reaction	2 (0.7)
Chest Discomfort	1 (0.4)
Disease Progression	1 (0.4)

SAEs of ADRs are 0.7% in the HAE studies population.

Of note is that in EDEMA3, for the five SAEs classified as HAE, there were three in the ecallantide group and two in the placebo. HAE attacks occurred on day 1 of treatment in the ecallantide group whereas both SAEs in the placebo patients occurred at later time points - day 2 and 7, both of which could be considered as representing a separate attack. Whether this classification of HAE in the ecallantide patients could represent misclassified allergic reactions was addressed by the applicant. Although the AEs of HAE in the ecallantide group were not HSRs, what was evident was that more AEs of HAE occur in the ecallantide versus the placebo group from days 0-2. This asymmetry at each time point (day 0, 1, 2) suggests lack or efficacy/exacerbation of the underlying HAE or rebound.

3 cases with anaphylactic/oid reactions, and 11 other cases with allergic symptoms identified as definitely/probably related to ecallantide are detailed below. 2 other cases are listed as possibly related.

Table 45. Events Identified as Potential Hypersensitivity Reactions in the Development Program

Pt ID	Symptoms	AE Coding (Symptoms/ Verbatim terms or Preferred SAE/AE terms)	Dose	Route	Time to Onset (min)	Severity	Relationship	Treatment	Antibody Status
8802003005	Dysphagia, pruritus, urticaria, edema, dyspnea, abdominal pain	Anaphylactoid reaction	1	IV	5	Severe	Probably	Epinephrine, antihistamines, hydrocortisone	NEG
8805051099	Erythema, pruritus, blood pressure 82/56	Anaphylactic reaction	19	SC	10	Severe	Definitely	Epinephrine, lorazepam, antihistamines	+ (ecall; Pichia IgE)
8820401009	Pruritus, erythema, dizziness, nausea, confusion	Anaphylaxis	5	SC	<1	Life-Threatening	Definitely	Epinephrine, antihistamines, methylprednisolone	+ (ecall; ecall IgE)
8805024097	Pruritus, erythema, dizziness, nausea, diaphoresis, feeling faint	Adverse drug reaction	6	SC	10	Severe	Probably	Epinephrine, antihistamines, hydrocortisone	+ (ecall)
8804013003	Rhinorrhea, itchy throat, shortness of breath	Acute allergic rhinitis with throat edema	1	IV	3	Severe	Probably	Epinephrine, antihistamines, corticosteroids	NEG
8805019001	eye erythema, eye swelling, urticaria, nasal congestion, rhinorrhea, sneezing, headache	Nasal congestion, urticaria localized, rhinorrhea, ocular hyperaemia, eye swelling, sneezing, headache	1	IV	2	Moderate	Definitely	None	NEG
8805050097	Abdominal discomfort, nausea, vomiting, throat itchiness, nasal congestion	abdominal discomfort, dyspepsia, nasal congestion, nausea, throat irritation, stomach discomfort, vomiting	1	IV	10	Mild	Probably	None	NEG

8814304010	Dyspnea, laryngeal edema, injection site erythema, rash	Dyspnea, laryngeal edema, injection site erythema, rash	7	SC	41	Mild to Moderate	Probably to Definitely	None	+ (ecall)
8820414001	Chest discomfort, flushing	Flushing and chest discomfort	16	SC	31	Moderate	Probably	Methylprednisolone, antihistamines, albuterol	+ (ecall)
8805054099	Headache, blurred vision, flushing, urticaria, pruritus, conjunctival redness, tearing, increased heart rate (172 bpm), increased blood pressure (152/100)	Adverse drug reaction	6	IV	1	Moderate	Definitely	Antihistamines	+ (ecall)
8814326002	Pruritus, nausea, injection site reaction	Pruritus generalized and injection site pruritus, nausea	4	SC	12	Mild to Moderate	Probably to Definitely	None	+ (ecall, Pichia IgE)
8804013007	Rhinitis	Rhinitis allergic	1	IV	Unk.	Moderate	Probably	Antihistamines	NEG
8805017018	Urticaria	Urticaria	2	SC	3.5 h	Severe	Possibly	Antihistamines, ranitidine, fresh frozen plasma, methylprednisolone (all before urticaria)	NEG (+ [ecall] at 28d f/u)
8820452001	Urticaria	Urticaria	10	SC	18	Severe	Probably related	Diphenhydramine	+ (ecall)
8814302003	Flushing, increased heart rate, increased blood pressure (all Dose 2 only); chest tightness, flu type body aches (Dose 3 only)	Dose 2: blood pressure increased, flushing, heart rate increased Dose 3: chest discomfort, pain	2, 3	SC	10	Mild	Possibly	None	+ (ecall)
8819443107	Pruritus, erythema, urticaria, angioedema	Pruritus, erythema, urticaria, angioedema	7	SC	5	Moderate	Definitely	Antihistamines epinephrine	+ (ecall)

Together with the 16 cases listed in table 45, an additional 2 cases were identified in the clinical study reports, leaving the total number of subjects who developed potential HSRs as 18. The incidence of systemic and/or severe allergic reactions following treatment with ecallantide is very high considered the limited safety database of 286 subjects.

It is noted that antibody positivity is not detected in all cases who had features of an allergic reaction based on their clinical features. In subjects with clinical features of a general allergic reaction, the negative results for the antibody tests could be due to too low a sensitivity, incorrect timing of the sampling or due to a direct action of the drug itself.

This very high immunogenicity has particular problems of rapid recognition in this patient group, and constitutes an unacceptable safety profile. An additional possible complication is that the hypotension which is typically associated with a systemic allergic response may be blunted by the reduction in bradykinin, thereby delaying recognition and treatment further.

Deaths

Two deaths have occurred in the ecallantide HAE program to date; neither death was related to study treatment.

In EDEMA1, Patient 8804022001 died in died of chronic renal failure secondary to rejecting his renal transplant 29 days after the administration of ecallantide. This patient had had dual nephrectomy and kidney transplant approximately 1 year prior to entering the study. The investigator stated that the patient began rejecting the transplanted kidney prior to treatment with ecallantide, and the event and subsequent outcome were assessed as unrelated to study medication.

Another subject was the victim of a homicide.

Laboratory findings

Hematology and chemistry

Overall, only a few clinically meaningful findings were observed in the hematology and chemistry analyses. In general differences were between baseline and endpoints were small and comparable between ecallantide treated and placebo treated patients. However there were some remarkable findings.

Reductions in lymphocyte count to threshold values (<5%) and reductions in neutrophil counts to threshold values (<30%) were seen. Also it can be understood that reductions are substantial.

The incidence of exceeding upper normal threshold values for ALT and AST is at least doubled in the *All HAE population* compared with the Double-Blind HAE SC Population. In the All HAE population 24 patients (8.6%) exceeded > 2.5 x ULN for ALT and 11 patients (3.9%) exceeded > 2.5 x ULN for AST. It is of interest to know if these elevations are dose dependent since patients can need more than one treatment within a short time. Information concerning the relation between incidence of elevated liver enzymes in relation to concomitant treatments, illnesses and HAE attack location is needed. The same is requested for Creatine kinase since 135 patients (48%) experienced exceeded Creatine kinase.

Table 46 Hematology Laboratory Change from Baseline: All HAE Studies Population

	Ecallantide (N=283)		
		Change from Baseline	
Laboratory Parameter	Baseline	Lowest	Highest
Lymphocytes (%)			
n[1]	268	268	268
Mean	25.0	-6.1	12.5
Median	24.8	-5.3	11.3
Std. Dev.	9.3	9.9	9.9
Minimum, Maximum	0.0, 50.6	-37.0, 15.2	-5.3, 55.0
Neutrophils (%)			
n[1]	268	268	268
Mean	66.8	-14.1	7.7
Median	66.7	-13.0	6.7
Std. Dev.	10.7	11.3	11.8
Minimum, Maximum	41.0, 99.0	-56.0, 8.0	-19.1, 46.0

Coagulation

Ecallantide is a selective, reversible inhibitor of plasma kallikrein. Ecallantide also inhibits plasmin activity up to approximately 10% at clinically relevant concentrations, and would thus be expected to have a (mild) antifibrinolytic effect. In HAE patients the maximum observed ecallantide plasma concentration following a 30 mg SC injection is approximately 0.6 µg/mL (ie, 3-fold lower). At concentrations of approximately 1 µg/mL, no effect on any intrinsic pathway clotting factors or on other measures of anticoagulation including aPTT, prothrombin time (PT), and thrombin time (TT) were observed in these studies.

Safety data did not reveal any clinical events that were definitively related to coagulopathies. Furthermore, there has been no safety signal for increased bleeding tendencies. Given ecallantide's intermittent dosing for acute HAE attacks and its short half-life, any coagulation abnormalities observed are expected to be transient.

The threshold values used for the coagulation analysis were: aPTT (>1.5×ULN); PT (>1.5×ULN); and TT (>30 seconds); see table 47.

Table 47: Incidence of Patients Reaching Threshold Values for Coagulation: Double-Blind HAE SC Population

		Ecallantide (N=100)			Placebo (N=81)		
Laboratory Test	**Threshold**	**N[a]**	**n[b]**	**(%)**	**N[a]**	**n[b]**	**(%)**
aPTT	>1.5 x ULN	96	0	(0.0)	74	1	(1.4)
Prothrombin Time	>1.5 x ULN	96	0	(0.0)	75	2	(2.7)
Thrombin Time	>30 sec	95	3	(3.2)	73	0	(0.0)

None of the out-of-range observations resulted in a clinically reported bleed or hemorrhage by any patient or any signs of increased bleeding risk.

Electrocardiogram

In preclinical development, ecallantide was shown to have no direct effects in standard cardiovascular assays. In the ecallantide clinical program, no formal QT/QTc studies were undertaken. Serial ECG monitoring in the EDEMA4 study was conducted. The ECG monitoring at the 4-hour time point is considered critical because the maximum plasma concentration (C_{max}) for ecallantide occurs at this time point.

Abnormal findings concerning QTc are displayed in table 48.

Table 48: Patients with at Least One Post-Dosing QTc Value of 450-479 msec, 480-499 msec, or >500 msec (Safety Population, Double-Blind Treatment)

	Treatment Group					
	Ecallantide (N=48)			Placebo (N=48)		
Laboratory Test	QTc (msec)			QTc (msec)		
Time Point	**450-479**	**480-499**	**>500**	**450-479**	**480-499**	**>500**
Patients with one or more post-dosing elevated QTc levels	3	0	0	3	1	0
Timepoint After Dosing						
2 Hours	1 (2.1)	0	0	3 (6.3)	0	0
4 Hours	3 (6.3)	0	0	3 (6.3)	0	0
7 Days	1 (2.1)	0	0	0	1 (2.1)	0

Source: Summary Tables 14.3.5.13.1; 14.3.5.13.2; 14.3.5.13.3; 14.3.5.13.4

In EDEMA3-RD no patient had a prolongation of the QTc interval at 2 hours post-dose that was clinically significant. No AEs related to QTc interval prolongation were reported. Abnormal findings in

other ECG intervals observed after treatment were assessed as either not clinically significant or, when clinically significant, were generally associated with underlying cardiac conditions observed on ECG at baseline. Two patients experienced sinus tachycardia as an AE. These findings are of particular interest because patients could receive multiple doses of ecallantide 30 mg SC.

The patients with prolongations in QT/QTc in EDEMA2 study, open label, are included in the analysis of the Completed HAE Studies and displayed in table 49.

Table 49 Incidence of Patients Reaching ECG Threshold Values

		Ecalantide (N=100) db		Placebo (N=81) db		Ecalantide (N=283) All HAE studies	
ECG Evaluation	threshold	N[a]	N (%)	N	N (%)	N	n (%)
Prolonged QTc Interval	> 500 msec	100	0	76	0	278	7 (2.5)
QTc Interval	> 60 msec change from baseline	100	1[c] (1.0)	76	0	278	15
PR Interval	> 200 msec	100	5 (5.0)	76	3 (3.9%)	278	18

[a] Number of patients with both a baseline and a post-base-line value
[b] Reflects the sum of patients in the normal to abnormal cell from the shift tables plus the number of patients in the abnormal to abnormal cell for whom the post-baseline value is more abnormal than the baseline value
[c] QTc = 65 msec change from baseline

In summary, there seems to be no relation between the QTc abnormalities and the applied doses. However, there is no convincing explanation for the QTc abnormalities seen in the open label study EDEMA2. Also PR interval prolongations occurred. Therefore further exploration of the medical histories and concomitant therapies are needed to assess the risk for QTc prolongations and PR interval prolongations.

Safety in special populations

A total of 31 paediatric patients between the ages of 10 and 17 have been treated with ecallantide, including 18 paediatric patients who have received one or more doses at 30 mg SC (84 acute attacks treated).

The pattern of TEAEs and SAEs were generally comparable between children and adults. In the placebo-controlled studies none of the 5 ecallantide treated paediatric patients reported a TEAE, compared with 36 (37.9%) of the 95 adult patients in these studies. In the placebo group the incidence was also comparable: 3 (30%) of the 10 paediatric patients vs 25 (35.2%).

Six paediatric patients reported a total of 14 SAEs, which are presented in Table 50. Two paediatric patients had events considered to be hypersensitivity reactions:

Table 50. Serious Adverse Events in Paediatric Patients

Patient	Serious Adverse Event	CTC Grade	Relationship to Ecallantide
8805024099	Hereditary Angioedema	2	Not Related
	Hereditary Angioedema	3	Not Related
8805019001	Cough	2	Definitely Related
	Nasal Congestion	2	Definitely Related
	Rhinorrhea	2	Definitely Related
	Sneezing	2	Definitely Related
	Throat irritation	2	Definitely Related
8805003004	Abdominal Pain diagnosed as Pancreatitis	3	Possibly Related
8805054099	Adverse Drug Reaction	2	Definitely Related
8804018004	Jaw Fracture	3	Not Related
8814301010	Concussion	2	Not Related
	Contusion	2	Not Related
	Skin Laceration	2	Not Related
	Hereditary Angioedema	3	Not Related

Immunological events

Overall, in all HAE studies, 45 (17.1%) patients seroconverted to anti-ecallantide antibodies (all classes), and 4 (2.1%) developed anti-ecallantide IgE antibodies. A total of 14 (8.0%) patients developed anti-P. pastoris IgE antibodies. A total of 16 of 197 patients tested (8.1%) have developed neutralizing antibodies to ecallantide.

Table 51. Antibody Seroconversion: All HAE Studies Population

	Post-Dose Ecallantide				
	Negative		Positive		Missing
Baseline	N	(%)	n	(%)	n
Antibodies to Ecallantide (All classes)					
Negative	212	(82.2)	42	(16.3)	3
Positive	0	(0.0)	4	(1.6)	0
Missing	6		3		13
IgE Antibodies to Ecallantide					
Negative	165	(98.8)	2	(1.2)	3
Positive	0	(0.0)	0	(0.0)	0
Missing	26		2		21
IgE Antibodies to *P Pastoris*					
Negative	134	(87.0)	8	(5.2)	3
Positive	0	(0.0)	12	(7.8)	0
Missing	27		6		29

Source: Supplemental Safety Analysis Table 10.3a.

Note: Percentages are based on the number of patients with both baseline and at least 1 post-baseline result.

The most serous safety concerns with ecallantide relate to the high rate of clinical reactions consistent with allergic responses. The presence of antibodies was not always detected in patients with a definite clinical allergic reaction. Furthermore there was missing data for many subjects.

Probability of seroconversion

For anti-ecallantide (all classes) antibodies, there is a steady increase in the probability of seroconversion with each treated episode through the twelfth. The probability of developing antibodies to ecallantide (all classes) antibodies after 12 HAE attacks is estimated to be approximately 68%.

Among patients for whom anti-ecallantide IgE antibodies were measured, no seroconversion was observed until the fourth attack. Although no further increase in the probability of seroconversion occurred from the sixth through the fourteenth episode, there are too few patients who were treated

for more than 8 HAE attacks to make any further conclusions. The probability of seroconverting to IgE anti-ecallantide antibodies after 8 HAE attacks is estimated to be approximately 12%.

For anti-*P. pastoris* IgE antibodies, there is an increase in the probability of seroconversion through the seventh episode. No further increase in the probability of seroconversion occurred after the seventh episode. Based on the curve, the probability of seroconverting to IgE anti-*P pastoris* antibodies after 7 HAE attacks is estimated to be approximately 30%.

There are too few patients who were treated for more than 8 HAE attacks to make any further conclusions.

This high predicted rate of seroconversion on repeated dosing is supportive of the serious objections relating to the safety of this product. The clinical reaction rate to ecallantide is high and may be under-represented in view of the difficulty in distinguishing a HAE attack from an allergic reaction. As HAE patients will require life long treatment at intermittent intervals, even those who have previously not had an allergic reaction to the product run an ever increasing risk of doing so in view of the high probability of seroconversion.

Safety related to drug-drug interactions and other interactions

N/A

Discontinuation due to AES

Two patients withdrew due to TEAEs in all HAE studies, Patient 8804024001 for lymphoproliferative disease (not related to study drug) and Patient 8805052097 for nausea (not related to study drug). Additionally, Patient 8805051099 experienced anaphylaxis (definitely related to study drug), and was disallowed from further treatments.

Post marketing experience

The company estimated that 133 patients have been treated in the US with 522 doses of ecallantide. Out of 34 reports 7 included terms such as pruritis or rash or hypersensitivity, and 21 reports contained terms such as ineffective or rebound or aggravated. The signals for condition aggravated/ineffective and allergic-type reports are of concern.

Discussion on clinical safety

The main safety concern is the very high incidence of allergic reactions including systemic and /or generalised allergic reactions, seen in patients who received ecallantide for the first time, and in those

who have had more than 1 injection. This combined with the high probability of seroconversion over repeated treatments, in a condition which can be confused with an allergic reaction and in which patients will require repeated intermittent dosing throughout life is a serious concern.

Conclusions on clinical safety

The safety profile of ecallantide is dominated by its high immunogenicity. Clinical reactions can develop in some cases on the fist administration and in others after repeated treatments. The consequences of these reactions can be confused with an HAE attack, thereby potentially leading to delay in treatments.

The incidence of systemic and/or severe allergic reactions following treatment with ecallantide is very high. This precludes its safe use in patients. This very high immunogenicity has particular problems of rapid recognition in this patient group, and constitutes an unacceptable safety profile. An additional possible complication is that the hypotension which is typically associated with a systemic allergic response may be blunted by the reduction in bradykinin, thereby delaying recognition and treatment further. Immunogenicity seems higher in the limited number of children tested.

Other safety concerns relate to effects of possible cross-reactivity of antibodies generated to ecallantide with the endogenous protein lipoprotein-associated coagulation inhibitor, effects of ecallantide on the clotting system, liver function, renal function, and interaction with drugs that affect the clotting system.

Pharmacovigilance system

The Applicant has provided documents that set out a detailed description of the system of pharmacovigilance (DDPS). A statement signed by the Applicant and the qualified person for pharmacovigilance, indicating that the Applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country has been provided.

Overall the CHMP considers that the Pharmacovigilance system as described by the Applicant generally fulfils the requirements and provides adequate evidence that the Applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country

The CHMP considers that the Pharmacovigilance system as described by the Applicant has the deficiencies (other concerns) as detailed in the LoQ

Risk Management plan

The applicant has submitted an updated RMP with the D120 responses, version 2.0, dated April 2011. The applicant is required to submit a further updated version of the RMP, having addressed all of the outstanding issues.

Safety Specification

Summary of safety concerns as presented in the updated RMP:

Important identified risks	Hypersensitivity reactions including anaphylaxis
	Prolongation of aPTT
	Immunogenicity
	Local tolerability/local reactions

Important potential risks	History of cardiac disorders
	Known allergy or antibodies to *P pastoris*
	Reproductive and developmental toxicities
Important missing information	Safety, tolerability and PK profile of ecallantide in patients under 12 years of age
	Safety, tolerability and PK profile of ecallantide in patients over 65 years of age
	Safety, tolerability and PK profile of ecallantide in ethnic origins other than Caucasian
	Safety, tolerability and PK profile of ecallantide in patients with a history of drug allergy
	Safety, tolerability and PK profile of ecallantide in patients taking concomitant alternative treatments for HAE.
	Safety, tolerability and PK profile of ecallantide in patients with hepatic or renal insufficiency
	Safety, tolerability and fetal effects when administered to pregnant women
	Safety, tolerability and effect on breastfeeding infants when administered to lactating women

The applicant has been asked again to further modify the list of important potential risks to include: 'autoimmunity against endogenous TFPI and possible risk of thrombosis', 'lack of treatment effect due to neutralising antibodies' and 'Risk of adverse reactions due to circulating immune complexes'.

The applicant has been asked to amend the important missing information on paediatric patients to "Safety, tolerability, and PK profile in all paediatric patients: safety information in patients below the age of 19 years, and under 15 in particular is limited."

Pharmacovigilance Plan

In addition to the ongoing phase 4 study in the US (LTOSS), the applicant has agreed to conduct a post-marketing safety study in the EU. The applicant has provided a short synopsis for a single-armed registry study.

The reasoning of the applicant for not including a comparator arm in the proposed EU study is considered weak and therefore the applicant is requested to confirm that they will include a comparator arm in the registry. The applicant should also consider the other points raised in this LoOI when designing the registry study.

The full study protocol must be submitted and approved prior to product launch; at this stage a revised synopsis, which takes into account all of the points raised in the assessment report must be provided in the updated RMP.

Evaluation of the need for a Risk Minimisation plan

Due to the high risk of hypersensitivity reactions with ecallantide, the applicant has proposed that a DHPC should be circulated at launch. Due to the risk that a DHPC may have a promotional effect, this proposal is not endorsed. In addition, a one-off measure such as a DHPC is not considered sufficient.

Instead, ongoing measures are required to inform all current and future prescribers of this risk and the applicant must produce educational materials that will be provided to all HCPs who prescribe ecallantide.

The educational materials must be submitted and approved prior to product launch. At this stage, the appropriate annex in the RMP must be updated with either a draft version of the educational program, or a description of its proposed key elements.

Risk Minimisation plan

Summary of the EU RMP

The RMP, proposed by the applicant and summarised below, requires updating both to bring it in line with the safety concerns in the above table, and to include the additional changes requested in the current LoOIs.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
Identified Safety Concern: Immunogenicity	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing monthly internal safety review meetings including QPPV) focused on reports of increased incidence of HSRs, anaphylaxis, and lack of product effect • Data collected via the ecallantide treatment registry will measure seroconversion and rates of HSR compared to other approved therapies • The Adjudication Panel will review Postmarketing adverse event reports that may be indicative of potential HSR. This review will better qualify and quantify events that may be indicative of HSRs, as well as discern between true hypersensitivity and worsening of the underlying HAE based on the criteria established by the National Institutes of Allergy and Infectious Disease (NIAID) and World Allergy Organization (WAO). The activities of this adverse event adjudication panel will be presented in the PSURs. • Postmarketing LTOSS will monitor antibody seroconversion rates and correlate with incidence of specific adverse reactions, including lack of product effect within the study population	SPC Section 4.4: Special warnings and precautions for use state that patients involved in the clinical studies with ecallantide developed antibodies to ecallantide. Rates of seroconversion increased with exposure to ecallantide over time. Overall, in all HAE studies, 57 (19.2%) patients seroconverted to anti-ecallantide antibodies (all classes), and 5 (1.7%) developed anti-ecallantide IgE antibodies. A total of 14 (4.7%) patients developed anti-*P. pastoris* IgE antibodies. A total of 18 of 212 patients tested (8.5%) have developed neutralizing antibodies to ecallantide. The long-term effects of antibodies to ecallantide are not known.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
Identified Safety Concern: Hypersensitivity reactions including anaphylaxis system complex terms include the following: adverse drug reaction, anaphylactic reaction, anaphylactoid reaction, erythema, flushing, hypotension, pharyngeal edema, pruritus, pruritus generalized, rash erythematous, rhinitis allergic, throat irritation, urticaria, urticaria localized, and wheezing	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) • Enhanced Pharmacovigilance including active follow-up for all potential hypersensitivity or anaphylactic reactions (reported events that meet the pre-defined symptom complex) by utilising a specialised questionnaire to obtain more complete and consistent information for each report of potential hypersensitivity in order to make more informed causality assessments and obtain sufficient information for further stratification • Monitor all reports of hypersensitivity or anaphylaxis for any increased frequency • Report significant findings in the PSUR with appropriate modifications to the product labeling as required • Data collected via the ecallantide treatment registry will measure seroconversion and rates of HSR compared to other approved therapies • The Adjudication Panel will review Postmarketing adverse event reports that may be indicative of potential HSR. This review will better qualify and quantify events that may be indicative of HSRs, as well as discern	• SPC Section 4.3: Contraindications states that ecallantide is contraindicated in patients with known hypersensitivity to ecallantide or any of its excipients • SPC Section 4.4: Special warnings and precautions for use states that ecallantide is only administered by a healthcare provider and patients must be observed for an appropriate period of time following administration • Package Leaflet • An emerging safety profile that differs from that already known with ecallantide may warrant changes to Section 4.3, Contraindications or Section 4.4, Special warnings and precautions for use, or additional risk minimisation activities may be warranted. Adverse events monitored over time and by volume will be reviewed internally on a monthly basis to identify potential safety signals and determine any changes to the benefit/risk assessment

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
	between true hypersensitivity and worsening of the underlying HAE based on the criteria established by the National Institutes of Allergy and Infectious Disease (NIAID) and World Allergy Organization (WAO). The activities of this adverse event adjudication panel will be presented in the PSURs. • Postmarketing Long Term Observational Study (LTOSS) will further quantify the true incidence of HSRs within the study population (refer to Annex 5)	
Identified Safety Concern: Prolongation of APTT	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) • Enhanced Pharmacovigilance including active follow-up for all potential reports of bleeding or coagulopathy by utilising a specialised questionnaire to obtain more complete and consistent information for each report bleeding in order to make more informed causality assessments and obtain sufficient information for further stratification • Postmarketing Long Term Observational Safety Study (LTOSS) will further quantify incidence of prolonged aPTT and other disorders of coagulation within the study	• SPC Section 5.1 Pharmacodynamic properties describe the known effect of ecallantide on aPTT

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
	population • Data from ecallantide treatment registry to determine rates of potential bleeding events in the targeted population • The Adjudication Panel will review Postmarketing adverse event reports that may be indicative of bleeding disorders	
Identified Safety Concern: Local tolerability / local reactions	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) • Postmarketing Long Term Observational Safety Study (LTOSS) will further quantify incidence of local reactions within the study population • Data from ecallantide treatment registry to determine rates of local reactions compared to other approved therapies in the targeted population	• SPC Section 4.8: Undesirable effects • Package Leaflet

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
Potential Safety Concern: Cardiac disorders	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) • Postmarketing Long Term Observational Safety Study (LTOSS) will further quantify incidence of cardiac disorders within the study population • Data from ecallantide treatment registry to determine comparative rates of events related to cardiac disorders in the targeted population	• SPC Section 4.8: Undesirable effects • Package Leaflet
Potential Safety Concern: Antibodies / known allergy to *P. pastoris*	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) • Data from ecallantide treatment registry to determine rates of potential related events in the targeted population	SPC Section 4.3: Contraindications Hypersensitivity to ecallantide or to any of the excipients. Known allergy to *P. pastoris*.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
Potential Safety Concern: Reproductive and developmental toxicities	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) • Enhanced Pharmacovigilance including active follow-up for all potential reports of reproductive or development toxicities by utilising a specialised questionnaire to obtain more complete and consistent information for each report bleeding in order to make more informed causality assessments and obtain sufficient information for further stratification • Data from the pregnancy registry in the ecallantide treatment registry will determine rates of potential reproductive or development toxicities in the targeted population	• SPC Section 4.6 Fertility, pregnancy, and lactation states that "There are no data from the use of ecallantide in pregnant women. Studies in animals have shown reproductive toxicity. ecallantide is not recommended for use during pregnancy." • SPC Section 4.6: Fertility, pregnancy and lactation Pregnancy There are no data from the use of ecallantide in pregnant women. Studies in animals have shown reproductive toxicity (see section 5.3). KALBITOR® is not recommended for use during pregnancy. Breastfeeding It is unknown whether ecallantide/metabolites are excreted in human milk. A risk to the newborns/infants cannot be excluded. A decision must be made whether to discontinue breast-feeding or to discontinue/abstain from KALBITOR® therapy taking into account the benefit of breast feeding for the child and the benefit of therapy for the woman.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
Missing Information: Safety, tolerability and PK profile of ecallantide in patients with renal, hepatic insufficiency	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing monthly internal safety review meetings including QPPV) focused on reports of hepatic and renal insufficiency with the objective of further categorising and classifying events reports to determine if the safety profile of ecallantide differs in patients with renal and hepatic insufficiency from the population proposed in SPC	• SPC Section 4.2 Posology and method of administration states that no information is available on patients with renal or hepatic impairment • An emerging safety profile that differs from that already known with ecallantide may warrant changes to Section 4.3, Contraindications or Section 4.4, Special warnings and precautions for use, of the SPC or additional risk minimisation activities. Adverse events monitored over time and by volume will be reviewed internally on a monthly basis to identify potential safety signals and determine any changes to the benefit/risk assessment
Missing Information: Safety, tolerability and PK profile of ecallantide in patients younger than 12 years old	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) • Enhanced Pharmacovigilance including active follow-up for all adverse event reports received in patients < 12 -< 16 years of age. Potential hypersensitivity or anaphylactic reactions (reported events that meet the pre-defined symptom complex) will be further investigated by utilising a specialised questionnaire to obtain more complete and consistent information for each report of potential hypersensitivity in order to make more informed causality assessments and obtain sufficient information for further	• SPC Section 4.1: Therapeutic indications states that ecallantide is indicated for symptomatic treatment of acute attacks of hereditary angioedema (HAE) in patients 12 years of age and older. • SPC Section 4.8: Undesirable Effects, Paediatric population, AE profile not dissimilar from adult AE profile • Package Leaflet • An emerging safety profile that differs from that already known with ecallantide may warrant changes to Section 4.2, Posology and method of administration, Section 4.3, Contraindications or Section 4.4, Special warnings and precautions for use, or additional risk minimisation activities may be warranted. Adverse events monitored over time and

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
	stratification to determine if the safety profile of ecallantide differs in patients under 16 years of age from the population proposed in SPC • DX-88/26, "A 3-Part Study to Evaluate the Pharmacokinetics, Safety, and Efficacy of Subcutaneous DX-88 in Prepubertal Paediatric Patients Experiencing Acute Attacks of Hereditary Angioedema" will be initiated in the EU	by volume will be reviewed internally on a monthly basis to identify potential safety signals and determine any changes to the benefit/risk assessment
Missing Information: Safety, tolerability and PK profile of ecallantide in patients over 65 years old	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing monthly internal safety review meetings including QPPV) with the objective of further categorising and classifying event reports to determine if the safety profile of ecallantide differs in patients over 65 years of age from the population proposed in SPC.	• SPC Section 4.2 Posology and method of administration states that limited information is available on patients older than 65 years of age • Package Leaflet • An emerging safety profile that differs from that already known with ecallantide may warrant changes to Section 4.3, Contraindications or Section 4.4, Special warnings and precautions for use, of the SPC or additional risk minimisation activities may be warranted. Adverse events monitored over time and by volume will be reviewed internally on a monthly basis to identify potential safety signals and determine any changes to the benefit/risk assessment

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimisation activities
Missing Information: Safety, tolerability and foetal effects when administered to pregnant women	• Routine Pharmacovigilance activities (PSURs, AE surveillance and reporting, literature surveillance, ongoing internal safety review meetings including QPPV) focused on pregnancy reports • A pregnancy registry, included in the design of the ecallantide treatment registry, will monitor patients who become pregnant following exposure to Kalbitor. Data collected from this registry will determine the need for any potential additional risk mitigation measures beyond the information contained in the approved Summary of Product Characteristics and Patient Information Leaflet	• SPC Section 4.6 Fertility, pregnancy, and lactation states that there are no data from the use of ecallantide in pregnant women and that ecallantide is not recommended during pregnancy. • All reported pregnancies are followed to term and parent/child data are entered in the global Pharmacovigilance safety database • Package Leaflet • If adverse event reports indicate an emerging safety profile infants of lactating patients, changes to Sections 4.4, Special warnings and precautions for use, and Section 4.6 of the SPC or additional risk minimisation activities may be warranted. Adverse events monitored over time and by volume will be reviewed internally on a monthly basis to identify potential safety signals and determine any changes to the benefit/risk assessment

ORPHAN MEDICINAL PRODUCTS

According to the conclusion of the COMP (Opinion dated 15 November 2002, (COMP/1920/02)) the prevalence of angioedema is approximately 2.0- 3.0 in 10,000 individuals in the EU.

Ecallantide, "recombinant inhibitor of human plasma kallikrein," is designated as an orphan medicinal product for the treatment of angioedema, entered into the Community register under the number EU/3/02/126 (December 2002). A similarity report was circulated for ecallantide on 20 May 2010.

BENEFIT RISK ASSESSMENT

Benefits

Ecallantide, kallikrein inhibitor, presents a novel mechanism in the treatment of angioedema due to lack of C1-inhibitor (HAE) compared to the currently approved options in the treatment of acute attacks which are plasma derived C1-inhibitor, recombinant human C1-inhibitor and the bradykinin B2 receptor antagonist icatibant.

During the development 286 patients have been treated with ecallantide in 5 clinical studies. In the 2 randomized, controlled, double-blind pivotal studies EDEMA3-DB and EDEMA4, 70 unique patients were treated with ecallantide 30 mg SC and 73 with placebo. In the supportive studies IV administration was principally studied whereas in EDEMA2 30 mg SC was also used.

The total patient population studied includes HAE patients from 10 years old of whom 34 were under the age of 18 years and is representative of the general HAE population.

Beneficial effects

Reduction of oedema is the aim of treatment since it will relieve laryngeal or airway narrowing, which may lead to asphyxiation. The time it takes for symptoms to respond to treatment is of primary importance in the treatment. Classically, the oedema and swelling develop gradually over several hours, increasing slowly for 12–36 h, and then subside after 2–5 days.

The times to onset of improvement, time to significant improvement and time to resolution are considered to be the clinically relevant measures of benefit. Of note, in the pivotal trials *time to significant improvement in overall response* was a secondary endpoint and *time to onset of sustained improvement in overall response* was a tertiary endpoint.

The secondary endpoint of *time to significant improvement in overall response* in EDMEA3 failed to reach statistical significance for the ITT-as-treated population and in addition the number of subjects censored in both the active and the placebo arms was high with 47.2% in the ecallantide group and 69.4% in the placebo group censored (ITT-as-treated population)

The secondary endpoint of *time to significant improvement in overall response* in EDMEA4 failed to demonstrate statistically significant difference between the active and placebo arms. In keeping with the results from EDEMA3, a high proportion of patients in each group were not included in this result as only 45.8% and 25.5% of subjects in the active and placebo arms respectively were defined as having significant improvement. This demonstrates that less than half the treated patients had a significant improvement using this outcome. The definition of significant improvement used was an overall responses assessment as "a lot better or resolved"

The tertiary endpoints of *time to onset of sustained improvement in overall results* seemed more favorable, in EDEMA3 with less patients censored. 72.2% and 27.8% of patients in the ecallantide and placebo groups respectively were defined as having sustained improvement (ITT-as-treated). The difference between the two groups reached statistical significance ($p<0.023$). However this was a tertiary endpoint and the definition of sustained improvement in this tertiary outcome measure included "a little better or a lot better or resolved" for a period of ≥45mins. The inclusion of "a little better" explains the higher proportion of patients with a positive response than for the secondary endpoint, but supportive measures such as IV fluids and NG tubes were allowed which may have impacted on this outcome. It is important to note that these patients could have been seen up to 12

hours after the onset of their attacks, a time at which some attacks may have reached their peak. In addition cases with a mild severity of attack were included, 8 in the ecallantide group and 6 in placebo.

The tertiary endpoints of *time to onset of sustained improvement in overall results* in EDEMA4 showed that 66.7% and 38.3% of subjects had sustained improvement, but the difference between the groups was statistically significant only with the log-rank test but not with the Wilcoxin test. This does not provide strong evidence of efficacy.

A second parameter, time to onset of overall response was neither in the separate studies nor in the integrated analysis statistically significant. In the third, significant improvement in overall response, a statistically significant difference in the time distribution was only seen in the integrated analysis.

Therefore the beneficial effects from treatment are not clear. Further clarification on "time to response" endpoints was provided in the day 120 responses. This showed that the effect of ecallantide varies with the location of the attack: best for abdominal, poor for laryngeal, particularly when laryngeal was assessed separately without reference to the overall response which would take into account concomitant symptom complexes, and no effect for peripheral attacks. Therefore the efficacy has not been show to be convincing and consistent even using the time to response parameters.

Uncertainty in the knowledge about the beneficial effects

With regard to laryngeal attacks, in the pivotal studies a statistically significant result in favour of ecallantide was not seen in the Kaplan Meier analysis of time to sustained response in patients.

Efficacy in paediatric patients ≥ 10 years old has not been demonstrated in controlled trials. Detailed information about the relation between dosing and responses is not found in the paediatric population.

Efficacy does not appear to be reduced in patients who were treated for multiple attacks over the longer term but the data is difficult to interpret.

There is a doubt as to whether the results of the pivotal studies are sufficiently similar to justify pooling of the time to response data.

In the pivotal studies the primary endpoints used were the Treatment outcomes score (TOS) in EDEMA3 and the mean symptom complex severity score (MSCS) in EDEMA 4. These PROs enable lack of efficacy such as worsening of concomitant symptom complexes and even development of new symptom complexes to contribute in a quantitative way to the PRO; rather than being recognised as a lack of efficacy and even a safety signal.

These outcome measures might be considered reflective of overall response in HAE for those with only a single symptom complex, and with a treatment that is not associated with exacerbation of the attack, development of a second attack or an allergic reaction.

However, the data from the studies provided raised serious concerns that these effects are seen with ecallantide; thereby making these PROs unsuitable for demonstration of efficacy for this product. Using these scales with their inherent limitations, efficacy is not considered to have been demonstrated for ecallantide, as the primary outcomes are problematic in view of their complexity and the possibility of masking lack of efficacy/ exacerbation of HAE/ rebound and allergic reactions.

These primary efficacy endpoints (TOS and MSCS) were based on the difference between patient reported outcome (PRO) scores at 4 hours after administration. Due to the lack of assessments of symptom severity or response to treatment between 4 hours and 24 hours an estimation of the time at which significant improvement was reached or at which an attack could be considered resolved was not

possible resulting in inadequate estimation of a median, confidence intervals and interquartile range (IQR).

Acknowledging the limitation of cross-trial comparisons there remains concern that the magnitude of the response in terms of time to beginning of response is small compared with placebo in the context of responses reported with other treatments available in the EU for HAE.

Risks

Across the development program, a total of 350 patients and healthy subjects were treated with 1387 doses of ecallantide in the clinical program, including re-challenge and compassionate use treatments.

Thirty one (31) paediatric patients received ecallantide of whom 6 were younger than 12 years of age at the time of their first exposure to ecallantide.

Unfavourable effects

The main safety concern is the very high incidence of allergic reactions including systemic and /or generalised allergic reactions, some of which could be life-threatening. Clinical reactions can develop in some cases on the first administration and in others after repeated treatments. The consequences of these reactions can be confused with an HAE attack, thereby potentially leading to delay in treatments.

The incidence of systemic and/or severe allergic reactions following treatment with ecallantide is estimated to be 4% in the clinical trials (conservative estimate). Using a less conservative estimate a value of ~6% is seen. This is considered very high. Two of the anaphylactic / anaphylactoid reactions were in children. This very high rate of clinical reactivity presenting as a generalised allergic reaction has particular problems of rapid recognition in this patient group, and constitutes an unacceptable safety profile.

Not all patients with a hypersensitivity reaction were positive for antibodies to ecallantide or to P Pastoris. Therefore there is no serological test identified that can exclude those more likely to develop a systemic reaction.

Antibody formation is high and a higher incidence of allergic AEs is seen in those patients who have or develop IgE antibodies to ecallantide and particularly to P pastoris. Currently, antibody development to ecallantide (any isotype) seems not to be strongly associated with an increased percentage of patients experiencing TEAEs, although there is a higher incidence of HSRs in those who seroconverted.

It can be expected that rates of seroconversion increases with exposure to ecallantide over time. This further strengthens the serious concerns regarding immunogenicity. As HAE patients require repeated treatments at variable intervals throughout life, the use of ecallantide in such an indication with the safety profile of this product strongly suggests that the current immunogenicity concerns will become more serious with repeated administration. In addition the similarity between anaphylaxis and a worsening of a HAE attack may even contribute to this risk since in practice it may be difficult to distinguish between the two.

It is of interest that aprotinin a 58 amino acid protease inhibitor derived from bovine lung which inhibits a variety of enzymes including kallikrein, was also associated with a high rate of serious allergic reactions, the risk of which increased on repeated exposure.

For ecallantide is not clear if the rate of seroconversions can be correlated with the concentration HCP although only traces of an allergen are sufficient to stimulate a reaction in a patient with specific IgE antibodies.

An additional safety concern relates to the increased incidence and earlier occurrence of AEs termed HAE in the ecallantide group compared with placebo. This reflects lack of efficacy or may reflect exacerbation of HAE, rebound or in some cases could be difficult to distinguish from an allergic reaction.

The most common TEAE is headache. Furthermore, ecallantide is mostly associated with gastro-intestinal side-effects notably nausea and diarrhoea. These types of TEAEs seem not to change with repeated dosing.

Uncertainty in the knowledge about the unfavourable effects

The relatively high incidence of hypersensitivity-like reactions and immunogenicity coinciding with serum antibodies (both IgE and IgG) against both ecallantide and Pichia proteins (HCP) is a major clinical concern. HCP and other host cell derived impurities (e.g. ß-glucans) may be related to these unwanted immunological responses. However, the applicant has indicated that immunogenicity is an intrinsic property of ecallantide being non-self to the human immune system due to the amino acid changes introduced to the TFPI Kunitz domain sequence and cannot be resolved as a quality issue. The question remains as to whether the drug substance therefore inherently results in, or contributes to, the anaphylactic/anaphylactoid reactions seen in clinical trials. Based on this clinical concern, the specification for Host cell derived impurities can not be considered clinically qualified.

In this regard it is not known if DX-88 induced hypersensitivity to Pichia related impurities (e.g. HCP and ß-glucans) leads to hypersensitivity for other medicinal products produced in Pichia.

An additional possible complication in cases who develop a systemic allergic reaction is that the hypotension which is typically associated with a systemic allergic response may be blunted by a reduction in bradykinin, thereby delaying recognition and treatment further.

The development of neutralising antibodies to ecallantide could manifest clinically as lack of efficacy or an immune complex-mediated reaction to the ecallantide. In addition neutralising antibodies could possibly cross-react with tissue factor pathway inhibitor (TFPI).

Although from the biopharmaceutical studies there did not appear to be a reduction in TFPI activity in neutralizing antibody positive samples this may be possible. If the concentration or activity of TFPI decreases this could lead to an increased risk of thrombosis. Indeed it is also possible that non-neutralising antibodies to ecallantide could cross-react on TFPI leading to reduced TFPI levels.

There is a uncertainty regarding prolonged QTc interval to more than 500 msec (seven patients), change from baseline in QTc interval of more than 60 msec (15 patients) and PR interval prolongation to more than 200 msec (eighteen patients), but no related AEs were reported. However, there is no convincing explanation for the QTc abnormalities and PR interval prolongations although they seem to happen more frequently in case of I.V. dosing. Therefore the risk concerning these findings should be further discussed before a definite opinion on this issue.

In the laboratory results a lowering of the lymphocyte and neutrophils counts and a rise in ALT and AST has also been observed. In the studies these were not associated with adverse events. There is no data to relate the transient decrease in the percentage of lymphocytes to treatment with ecallantide and these changes are not considered to be clinically significant. Some of the increases were extremely high and were seen on multiple occasions where ecallantide was administered. More data is needed on the attack location and also on concomitant medication, concomitant illnesses or other relevant factors and their temporal relation to the increases in AST and ALAT for a final assessment on the increases in ALT and AST issues.

Concerning the paediatric population, the inclusion of the number of patients was compliant with the PIP. The data in children aged 12 to 16 years has been obtained over different dosages with different routes of administration.

PK data indicates that with the fixed dose of 30 mg SC, C_{max} and AUC0-4h was considerably higher in patients <18 years of age, suggesting that a weight based dosing regimen may result in more comparable exposure to ecallantide in paediatric and adult patients. More PK data are requested together with a new popPK model. At the moment the 30 mg SC dose has not been sufficiently justified in this age group.

With regard to the compliance check of the PIP only one comment was made by the PDCO: the PK data per category should be detailed.

Ecallantide has a short half-life (~2 hrs) time and it is not intended to be used as a preventive treatment. To develop a new attack after treatment for a HAE attack is unusual with current therapies but there have been more AEs of HAE in the ecallantide treated subjects than in the placebo group in the clinical programme. This is of concern because it may reflect lack of efficacy/rebound then the posology is not optimal. The choice of dose and route remain concerns for ecallantide.

Balance

The balance is for insufficient evidence of a clinically relevant efficacy.

A clinically relevant beneficial effect for *time to significant improvement in overall response*, a secondary endpoint, has not been adequately demonstrated in either of the two pivotal trials.

A beneficial response in the integrated analysis was seen for time to significant improvement in overall response, but this is not considered supportive, as in the pivotal trials this was a tertiary endpoint and did not show clear evidence of statistical significance in both trials. In addition there were factors, such as severity of attack and supportive interventions that could have contributed to the results obtained for this endpoint.

Hypersensitivity and in particular anaphylactic reactions occurred in 16 and 4 patients respectively, not all of whom had developed antibodies to either ecallantide or to P Pastoris. However those who did develop antibodies, particularly IgE antibodies to P Pastoris have a higher incidence of HSR than those who were antibody negative. It can be expected that rates of seroconversion increase with exposure to ecallantide over time with possibly a higher risk of a hypersensitivity reaction.

There is a uncertainty regarding increases in ALT and AST and also QTc interval prolongation and PR interval prolongation. Since there is no convincing explanation the risk concerning these findings should become more clear.

Efficacy data in paediatric patients ≥ 10 years old are indicative of efficacy but the results are not conclusive and there remain questions regarding the dosing. The data indicate some effect of weight on the pharmacokinetics of ecallantide and on the efficacy. This effect of weight on pharmacokinetics and efficacy should be further explored by popPK analysis (dose response curves, Kaplan-Meier curves) in adolescent and adult patients.

Importance of favourable and unfavourable effects

The main risk is that if a serious allergic reaction constitutes a major problem in terms of accurate diagnosis in this patient group. This may lead to appropriate treatment delays.

However as subjects also developed serious systemic reaction in the absence of seroconversion, this highlights the fact that there is no way of predicting these reactions nor of excluding patients who are likely to develop a SR. It is highly probable that there is more than one mechanism underling the high rate of HSRs with ecallantide; only some of which are related to seroconversion.

Overall, in all HAE studies, 45 (17.1%) patients seroconverted to anti-ecallantide antibodies (all classes), and 4 (2.1%) developed anti-ecallantide IgE antibodies. A total of 14 (8.0%) patients developed anti-P. pastoris IgE antibodies. A total of 16 of 197 patients tested (8.1%) have developed neutralizing antibodies to ecallantide.

In addition the likelihood of developing antibodies to ecallantide over repeated administrations at variable intervals due to the episodic and unpredictable nature of the attacks, means that patients will always be at risk of this even if they have tolerated ecallantide in the past.

Seroconversion and concomitant hypersensitivity and anaphylaxis cover the most important serious adverse event. Anaphylaxis as a life threatening condition was seen in adults and children.

Benefit-risk balance

The overall B/R of ecallantide in the treatment of HAE is negative in view of the lack of demonstration of clinically convincing and consistent efficacy in the two pivotal trials and the unfavourable safety profile. The adverse effects are dominated by concerns regarding the high rate of allergic reactions in such a relatively small safety data base and the increasing rate of seroconversion over repeated administrations to both the product itself and to P.pastoris, particularly IgE antibodies.

Discussion on the benefit-risk assessment

The pivotal trials used PROs that are considered to inadequately reflect failure of treatment. For the secondary endpoints in both trials, *time to significant improvement in overall response* was included, but failed to reach statistical significance.

The tertiary endpoints of *time to onset of sustained improvement in overall results* seemed more favorable, in both trials. As this is a tertiary endpoint these results are not considered sufficient to provide robust evidence of efficacy.

When the time to response analysis was conducted for different anatomical locations, the results were surprising in that efficacy was shown for abdominal attack, efficacy was weak for laryngeal attacks and efficacy was not demonstrated for peripheral attacks. Such variable results for efficacy at different anatomical locations is not consistent with an effective systemic therapy for HAE.

The most important adverse events are generalized and/or severe allergic reactions. The applicant provided details of three cases considered by the investigator to have an anaphylactic/oid reactions

In the whole clinical programme there were 18 cases, of which 6 were safely re-treated leaving 12 cases with potential HSRs. However these 6 cases are important to consider also as some had systemic reactions consistent with HSR of an anaphylactic or anaphylactoid nature.

With a small safety database these numbers provide a serious safety signal relating to the product itself. The high rate of antibody development both to the product itself and also to the HRIs from P.pastoris, together with the increasing seroconversion rate expected following repeated administration, combine to make the risks to outweigh the unconvincing evidence provided in support of efficacy for ecallantide.

Concerning the paediatric population, the inclusion of the number of patients was compliant to the PIP. The data in children aged 12 to 18 years has been obtained over different dosages with different routes of administration. However, PK data indicates that with the fixed dose of 30 mg SC, C_{max} and AUC0-4h was considerably higher in patients <18 years of age. A weight based dosing regimen may be result in more comparable exposure to ecallantide between children and adults. At the moment the 30 mg SC dose has not been sufficiently justified in this paediatric age group. Since the number of included children is low the safety data are limited. And in spite of these limited number still 2 children experienced a hypersensitivity reaction, although this can be due to chance occurrence. Overall the safety data are insufficient to overcome the concerns regarding insufficient dose finding in combination with the PK data with respect to the higher exposure in children than in adults.

Conclusions

The overall B/R of ecallantide is negative.